1/59

EcoR I   Sma I
Sac I   BamH I
Kpn I   Xba I
                     Sal I
                     Pst I
Cla I   Sph I
                     Hind III

EcoR V   Kpn I   I3

                11k

gpt   R-tk

                                    ori

pgpt ATA 18

L-tk   5300 bp

Xba I   Bal I

Nhe I   Amp

                                   Pvu I

F1(-)   Sca I
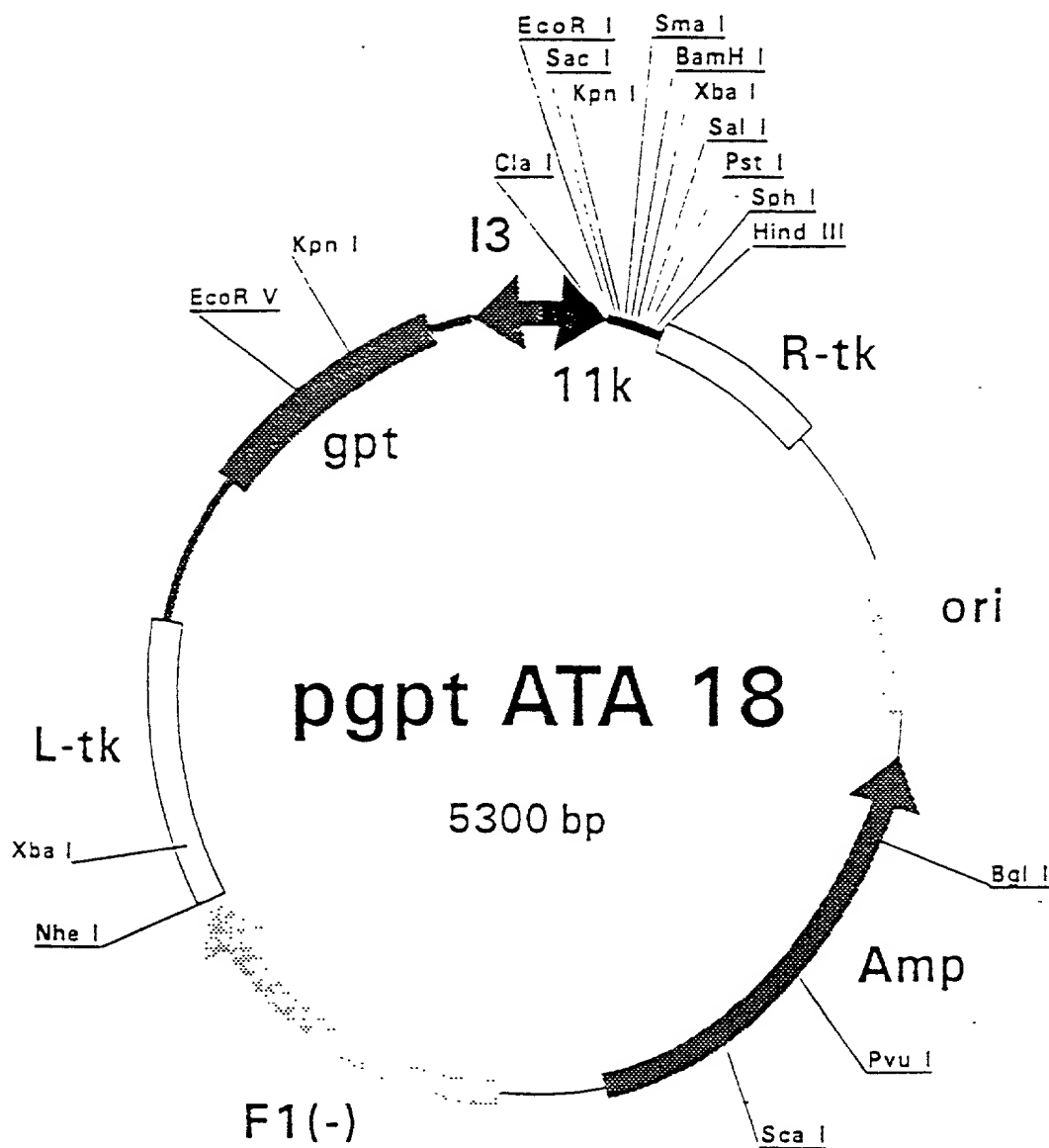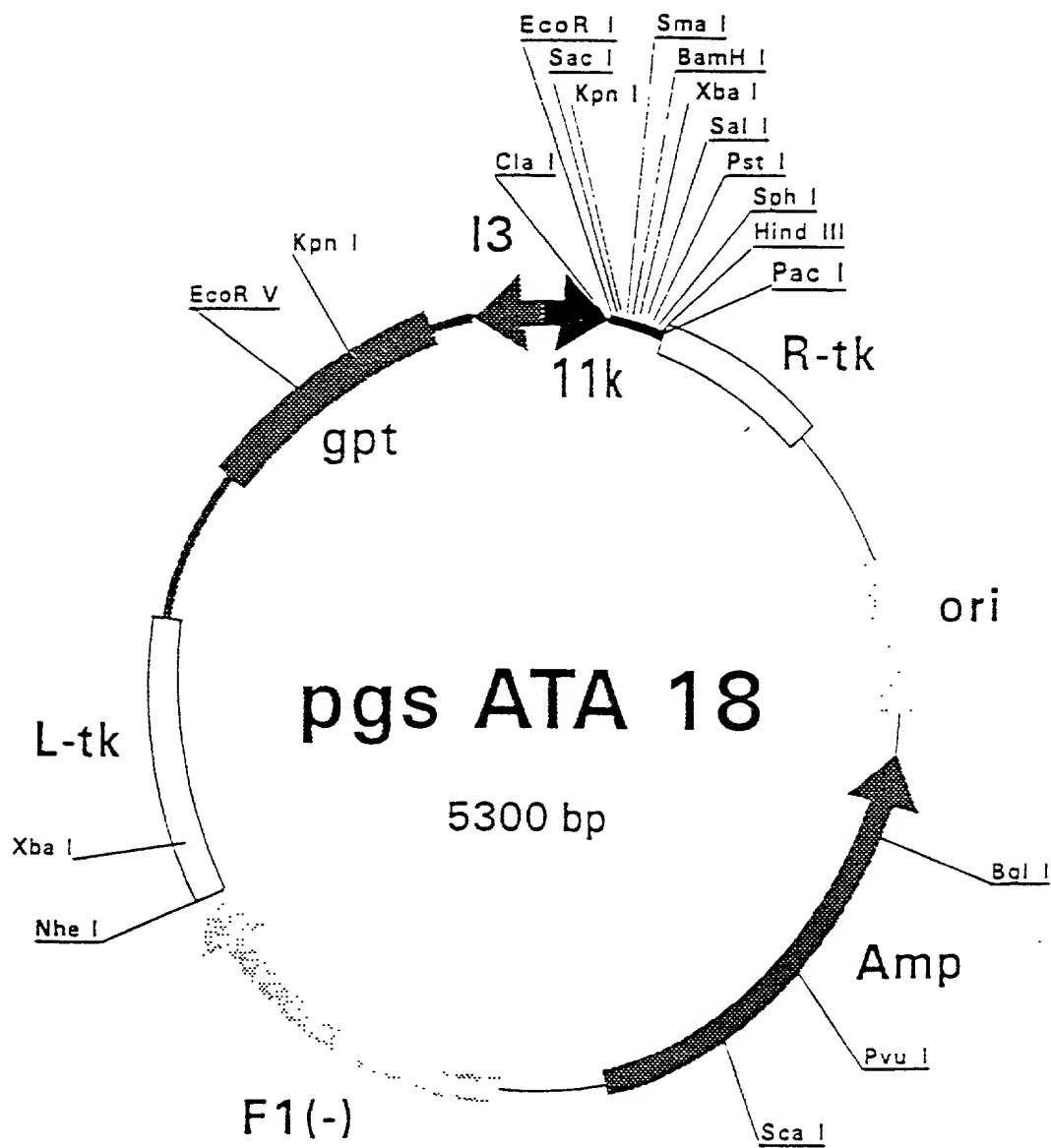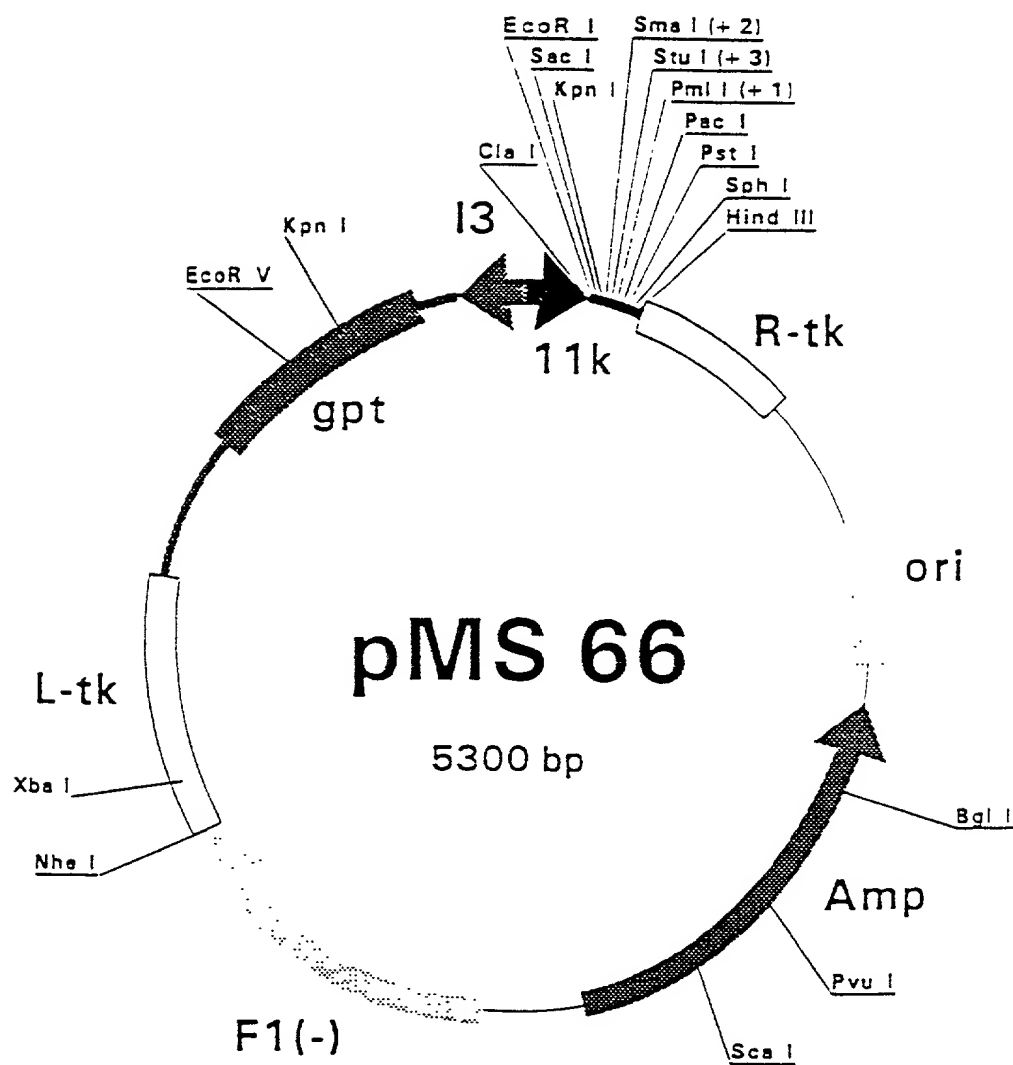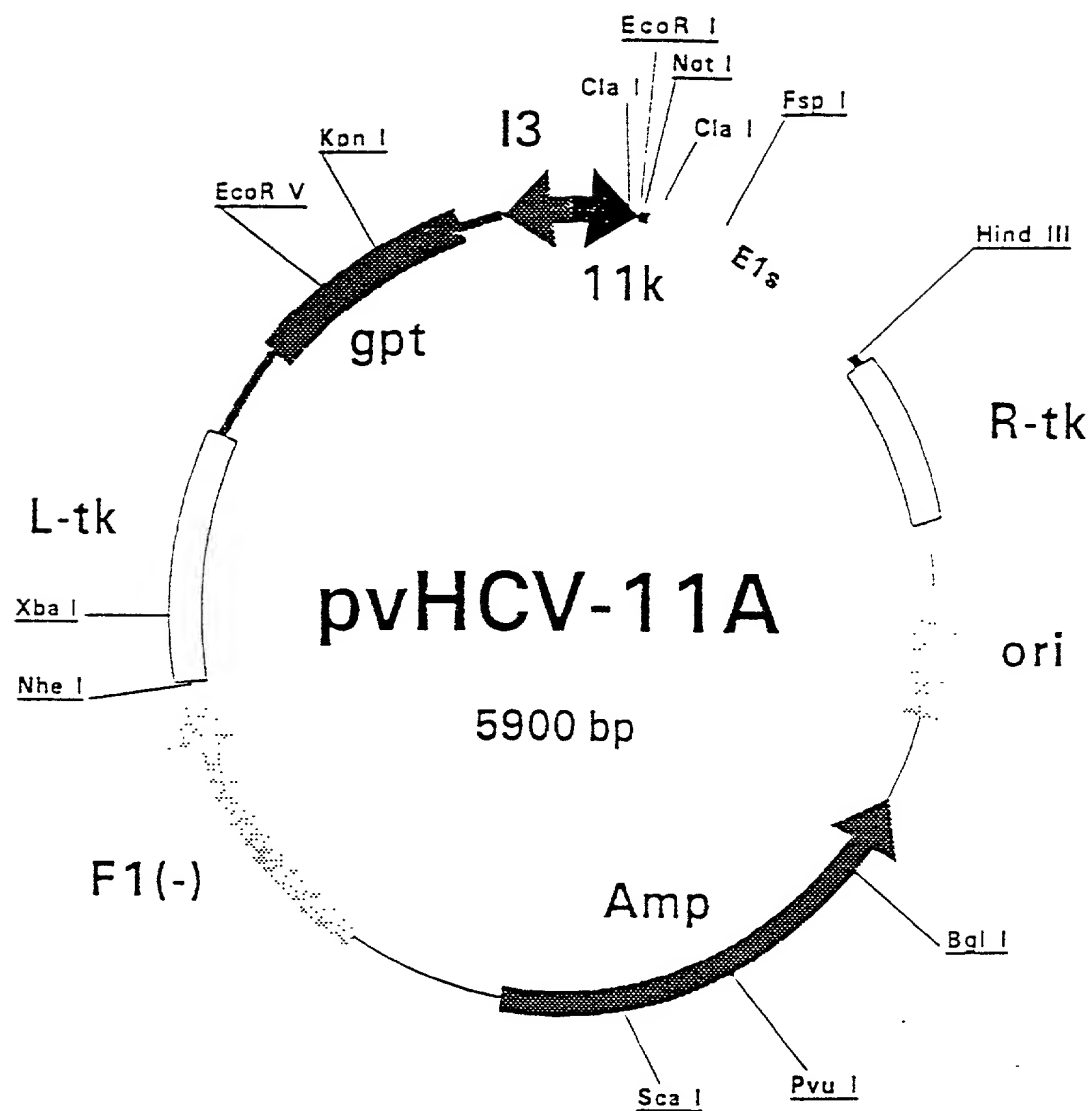
FIGURE 1

FIGURE 2

FIGURE 3

4/59

FIGURE 4

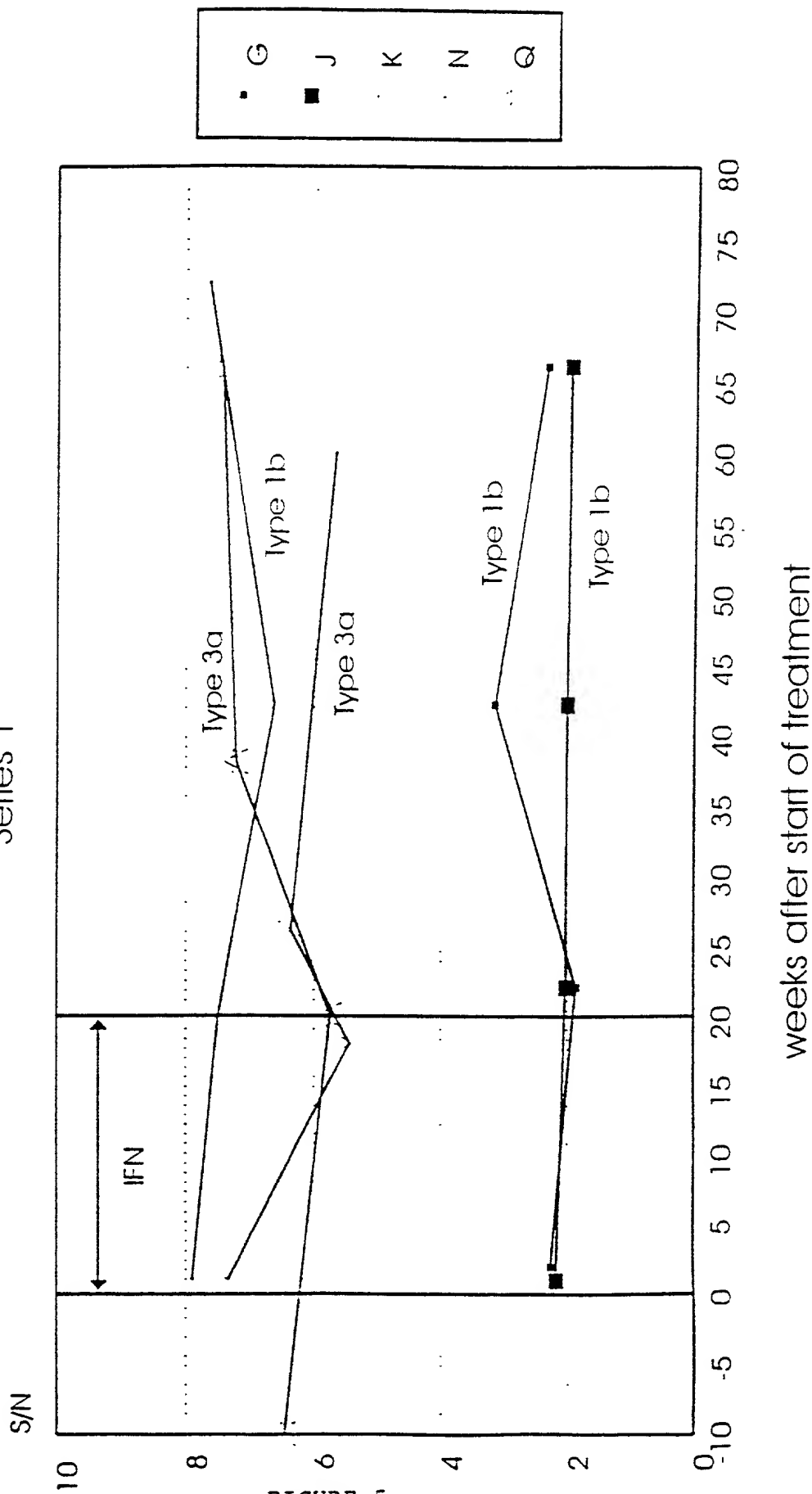## Anti-E1 levels in NON-responders to IFN treatment

Series 1

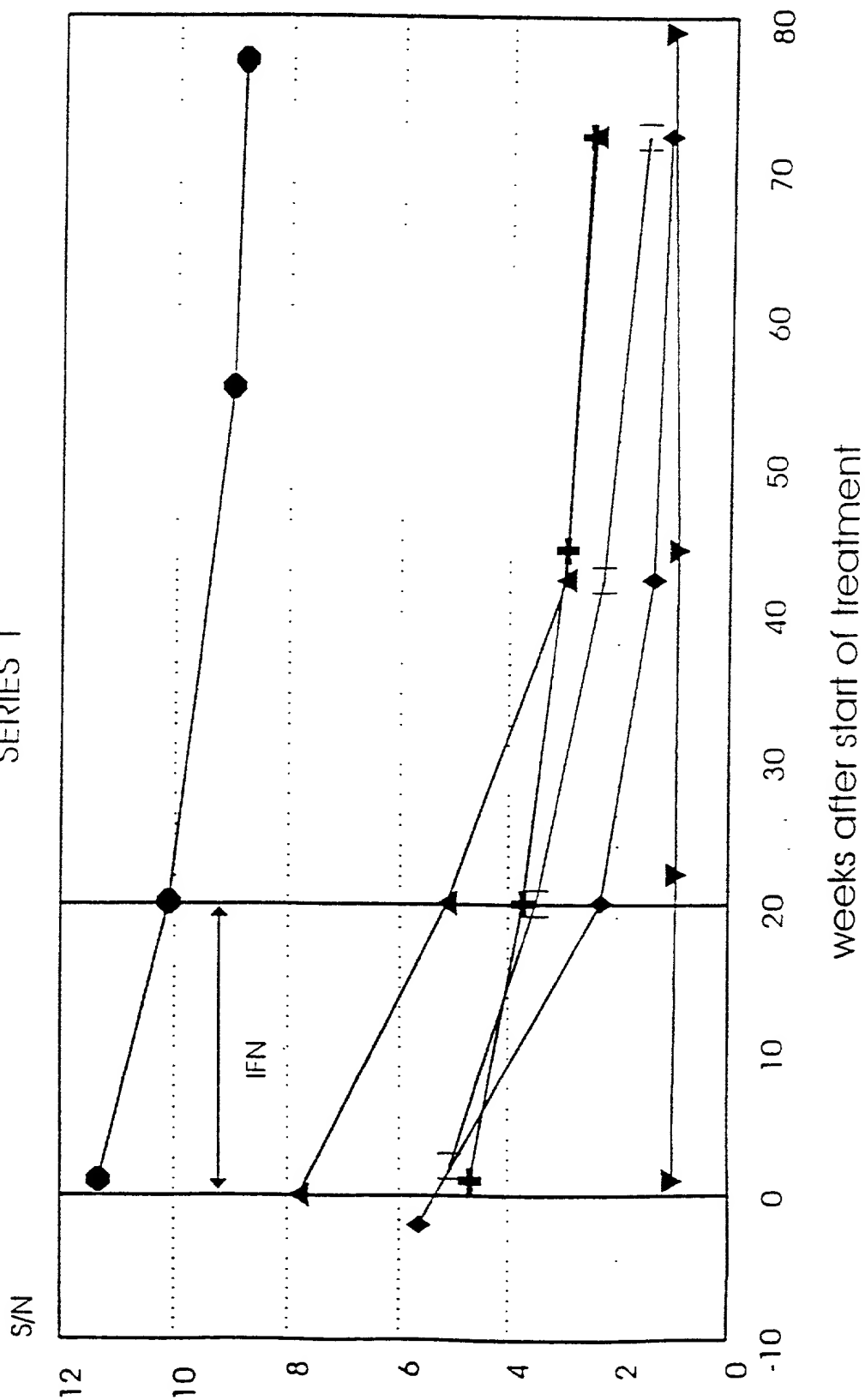

FIGURE 5

Anti-E1 levels in RESPONDERS to IFN treatment

SERIES 1

Anti-E1 levels in patients with COMPLETE response to IFN

SERIES 2

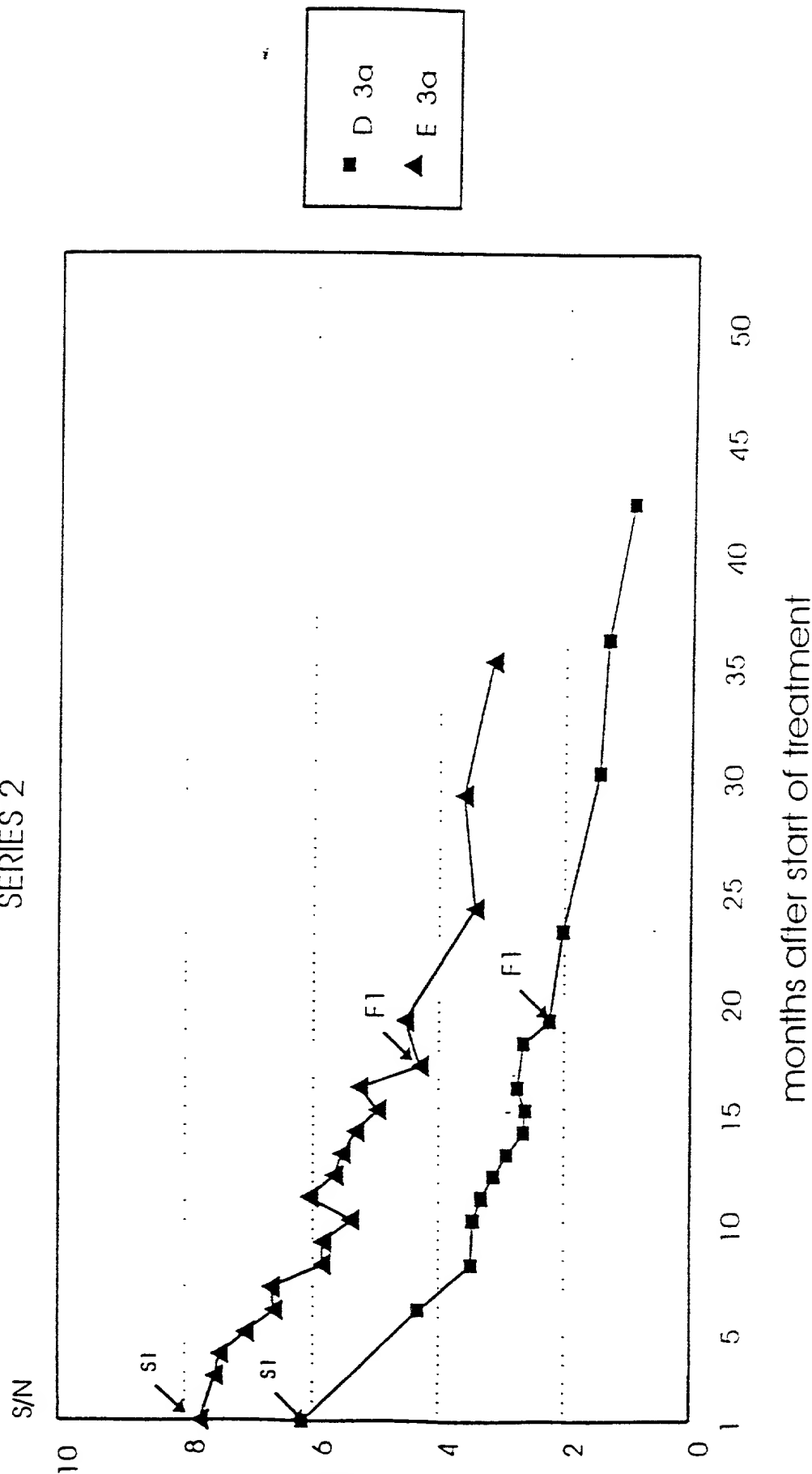FIGURE 7

Anti-E1 levels in INCOMPLETE responders to IFN treatment
SERIES 2

FIGURE 8

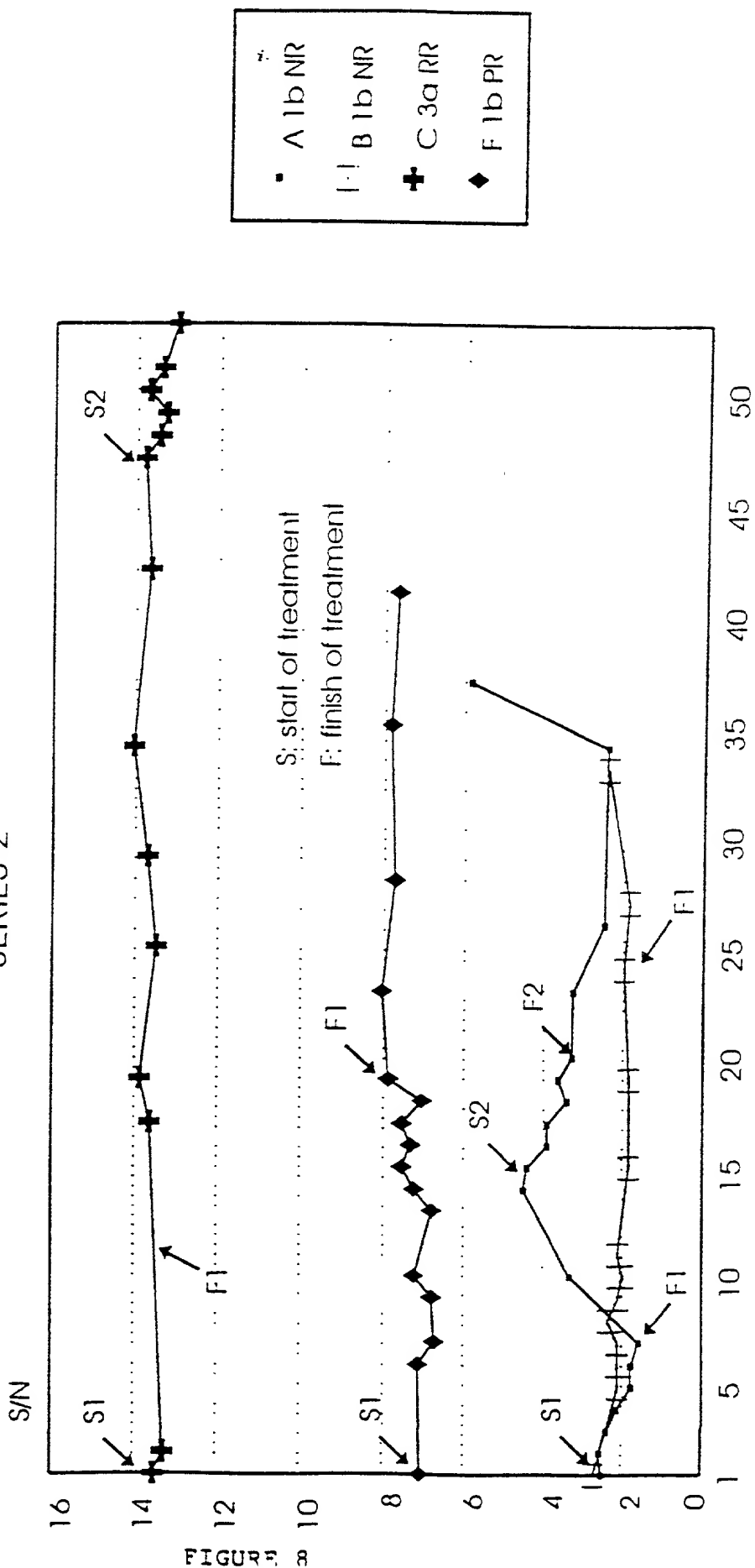Anti-E2 levels in NON-RESPONDERS to IFN treatment

SERIES 1

Legend:
- G 1b
- J 1b
- K 1b
- N 3a
- Q 3a

weeks after start of treatment

S/N

IFN
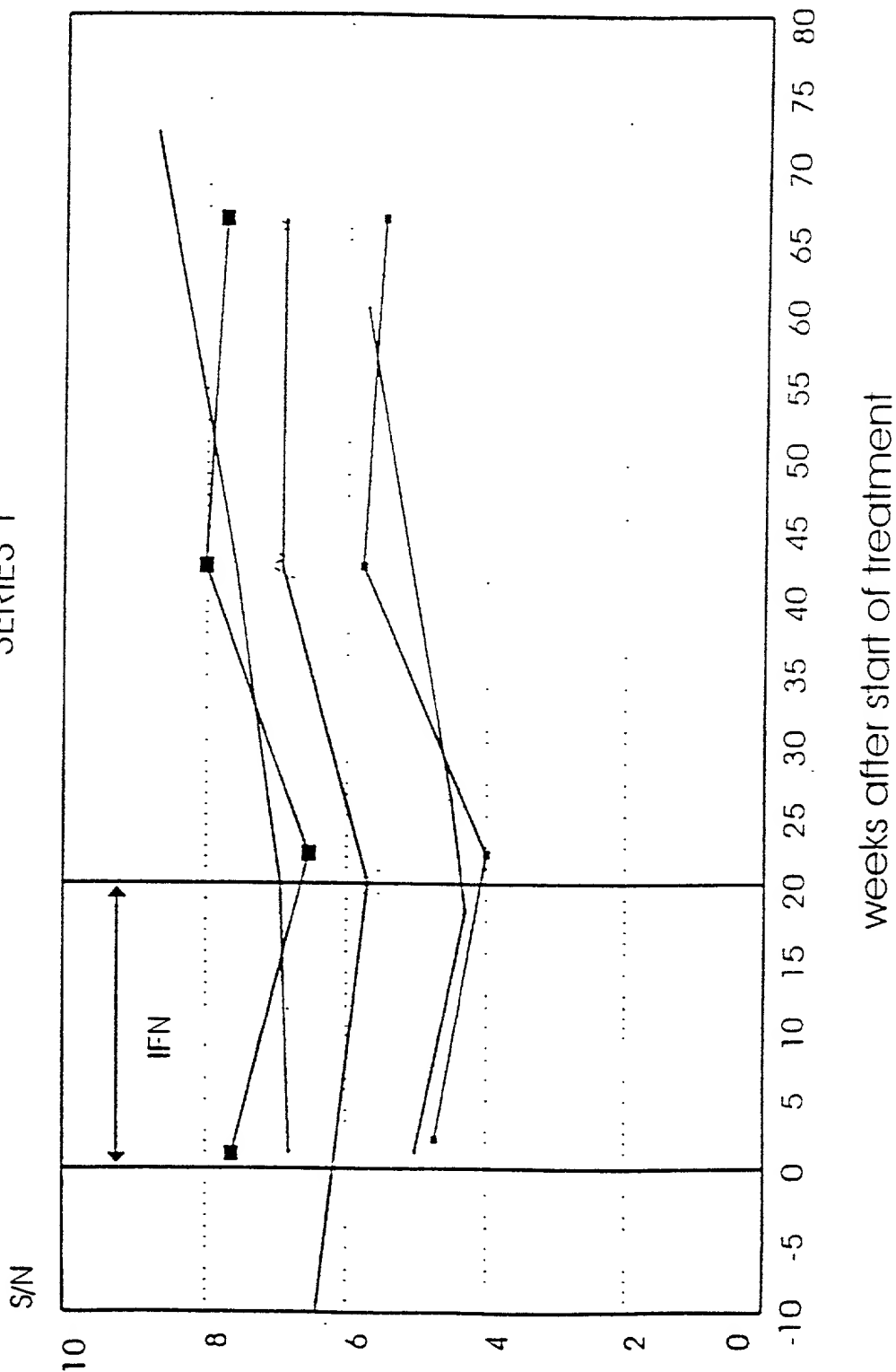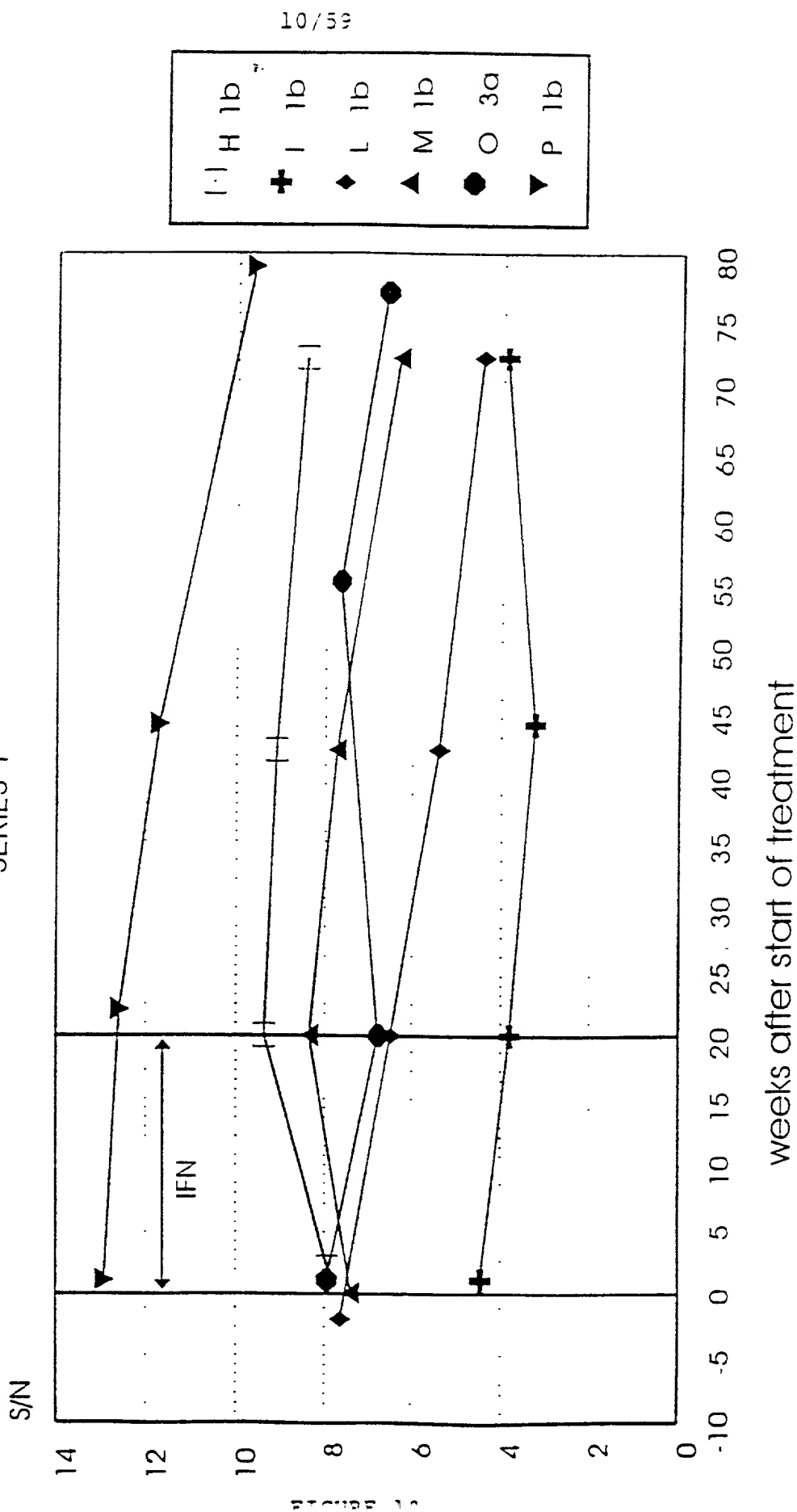
FIGURE 9

FIGURE 11

Anti-E2 levels in INCOMPLETE responders to IFN treatment

SERIES 2

FIGURE 11

Anti-E2 levels in COMPLETE responders to IFN treatment

SERIES 2

■	D 3a CR
▲	E 3a CR

F : finish of treatment
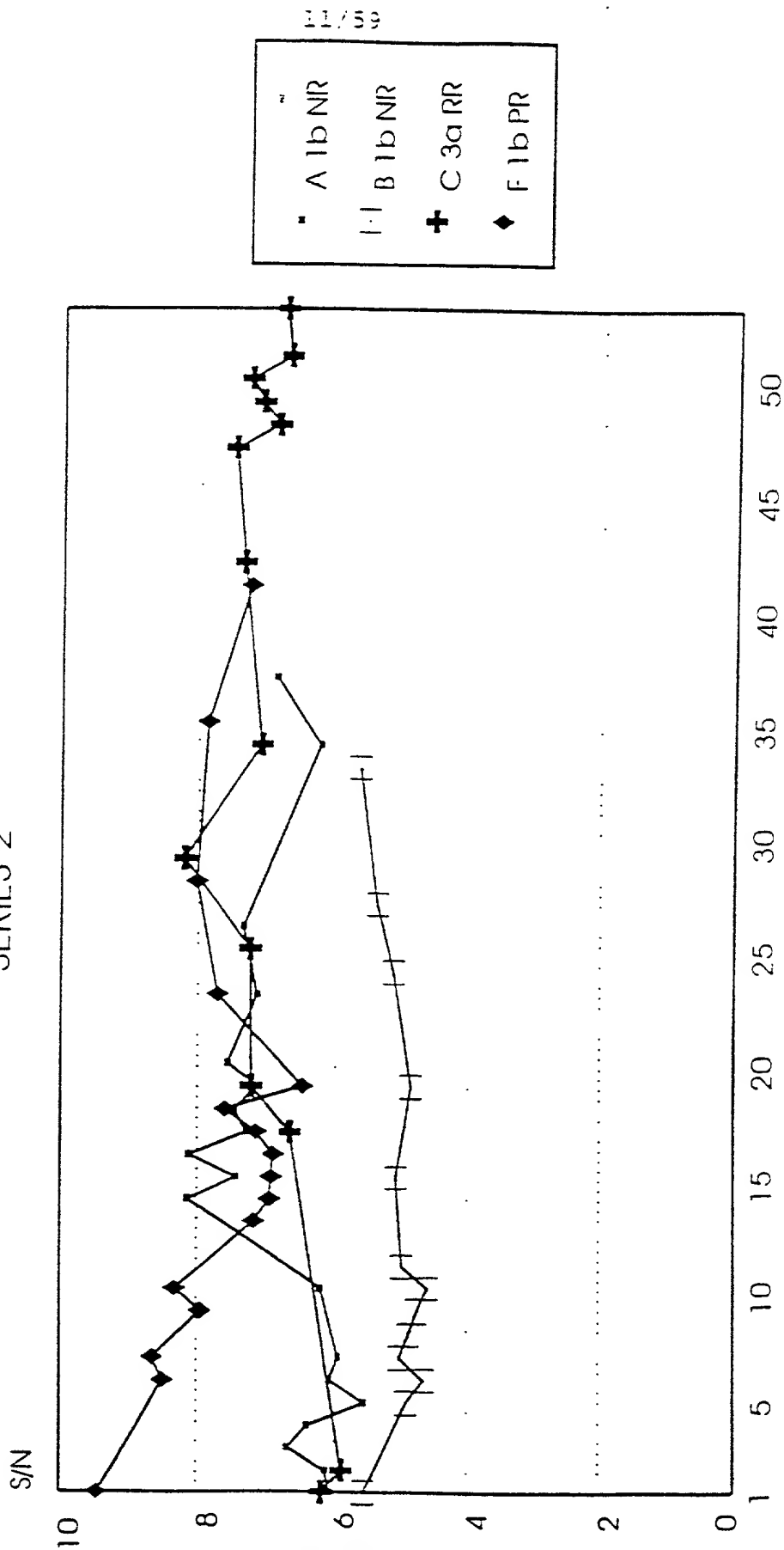
months after start of treatment
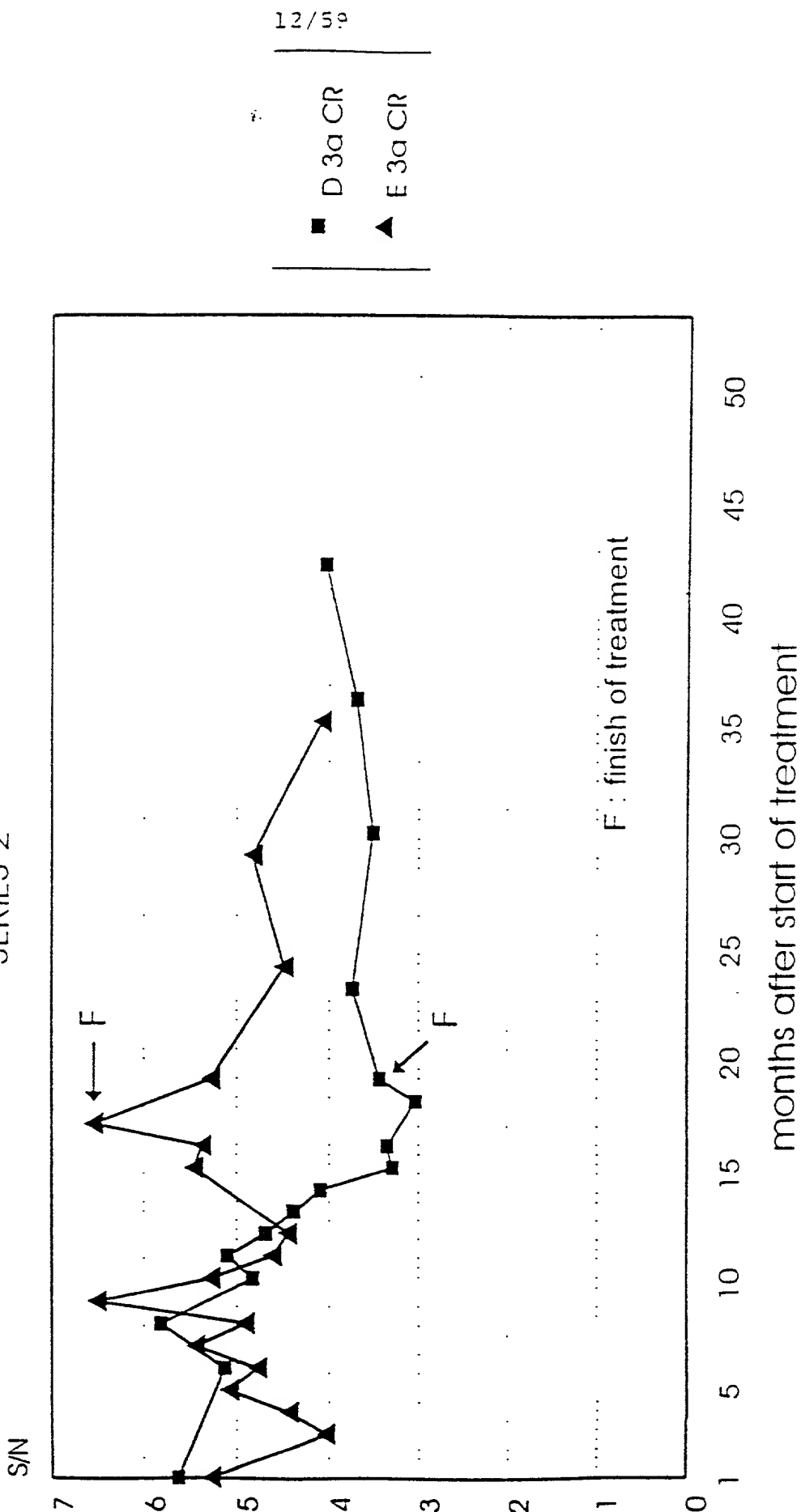
FIGURE 12

FIGURE 13

Human anti-E1 reactivity competed with peptides
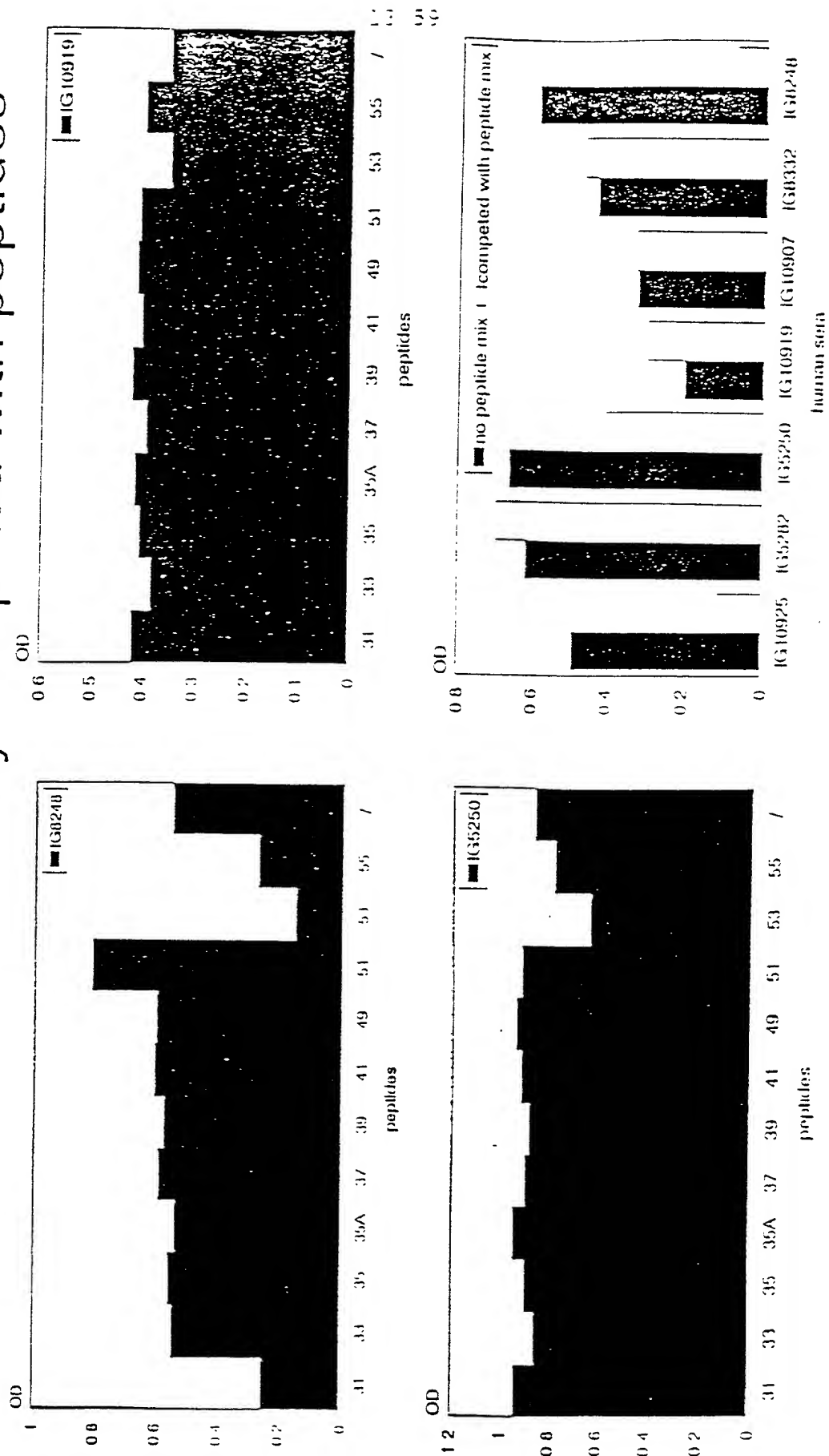
FIGURE 14

Competition of reactivity of anti-E1 Mabs with peptides

Anti-E1 (epitope 1) levels in NON-RESPONDERS to IFN treatment

SERIES 1

Legend:
- G 1b
- ■ J 1b
- K 1b
- N 3a
- Q 3a

S/N

weeks after start of treatment

IFN
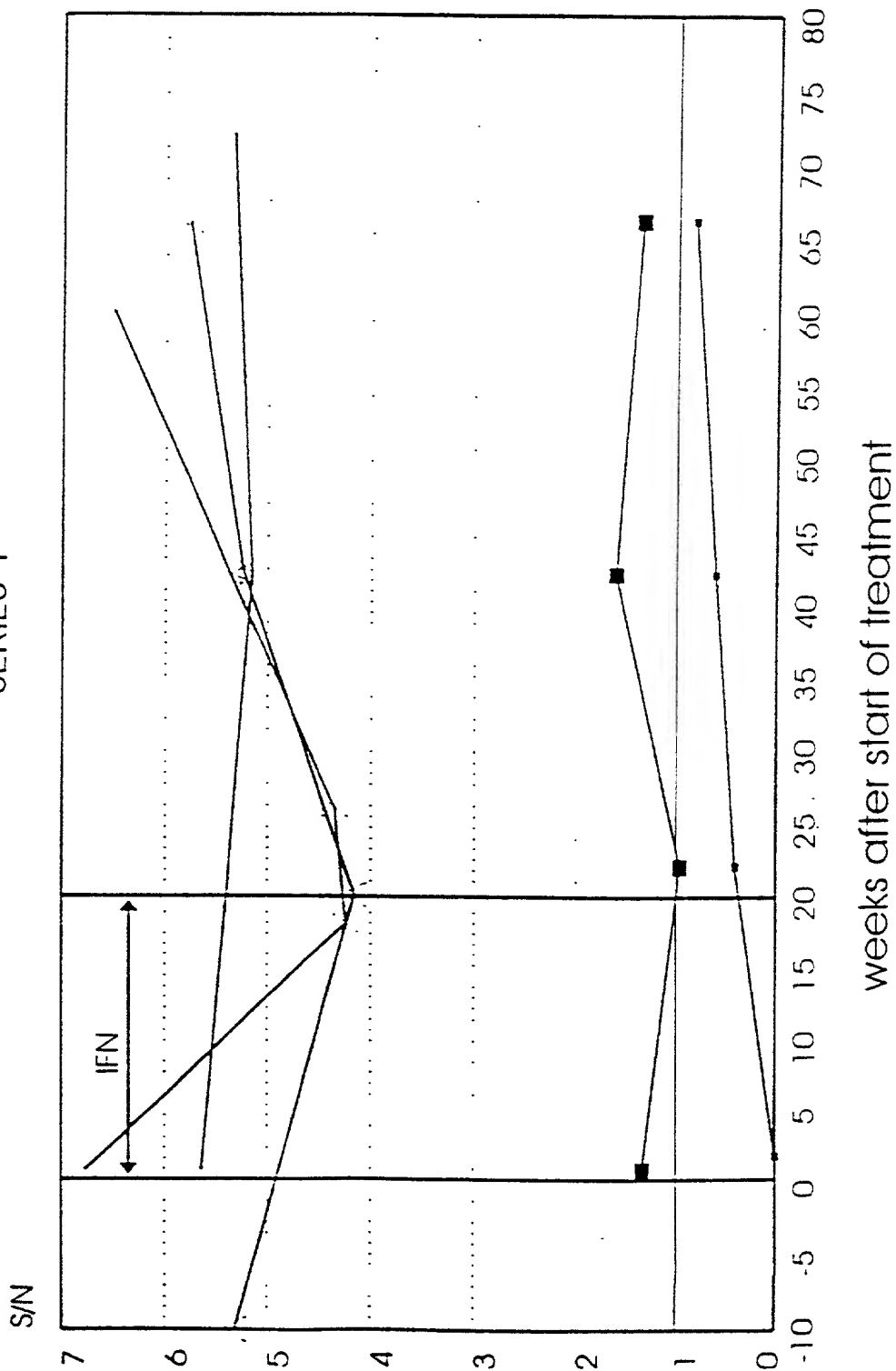
FIGURE 15

Anti-E1 (epitope 1) levels in RESPONDERS to IFN treatment

SERIES 1

nti-E1 (epitope 2) levels in NON-RESPONDERS to IFN treatment

SERIES 1

■	G 1b~
■	J 1b
	K 1b
	N 3a
	Q 3a

weeks after start of treatment

S/N

IFN

Anti-E1 (epitope 2) levels in RESPONDERS to IFN treatment

SERIES 1

weeks after start of treatment
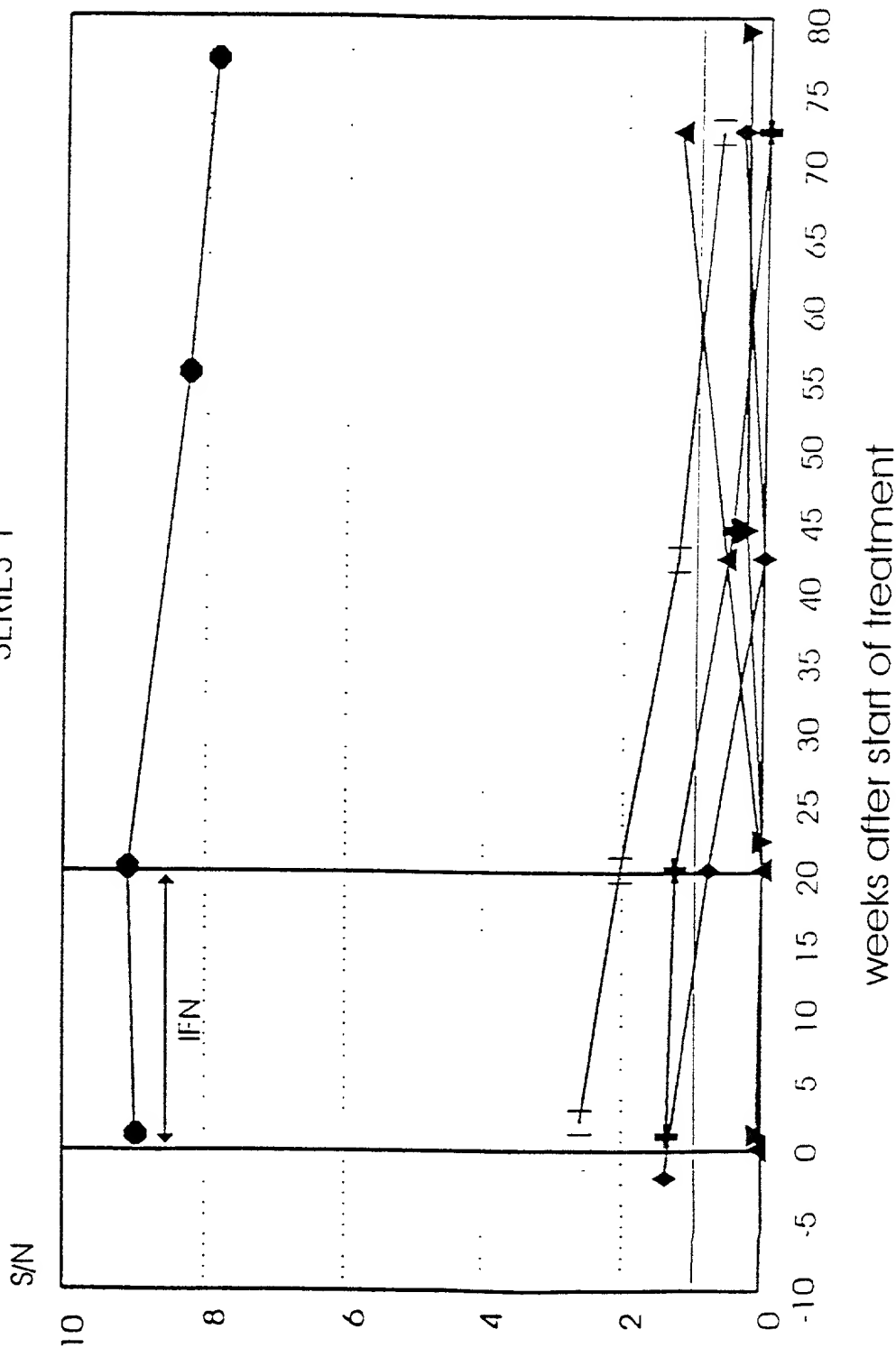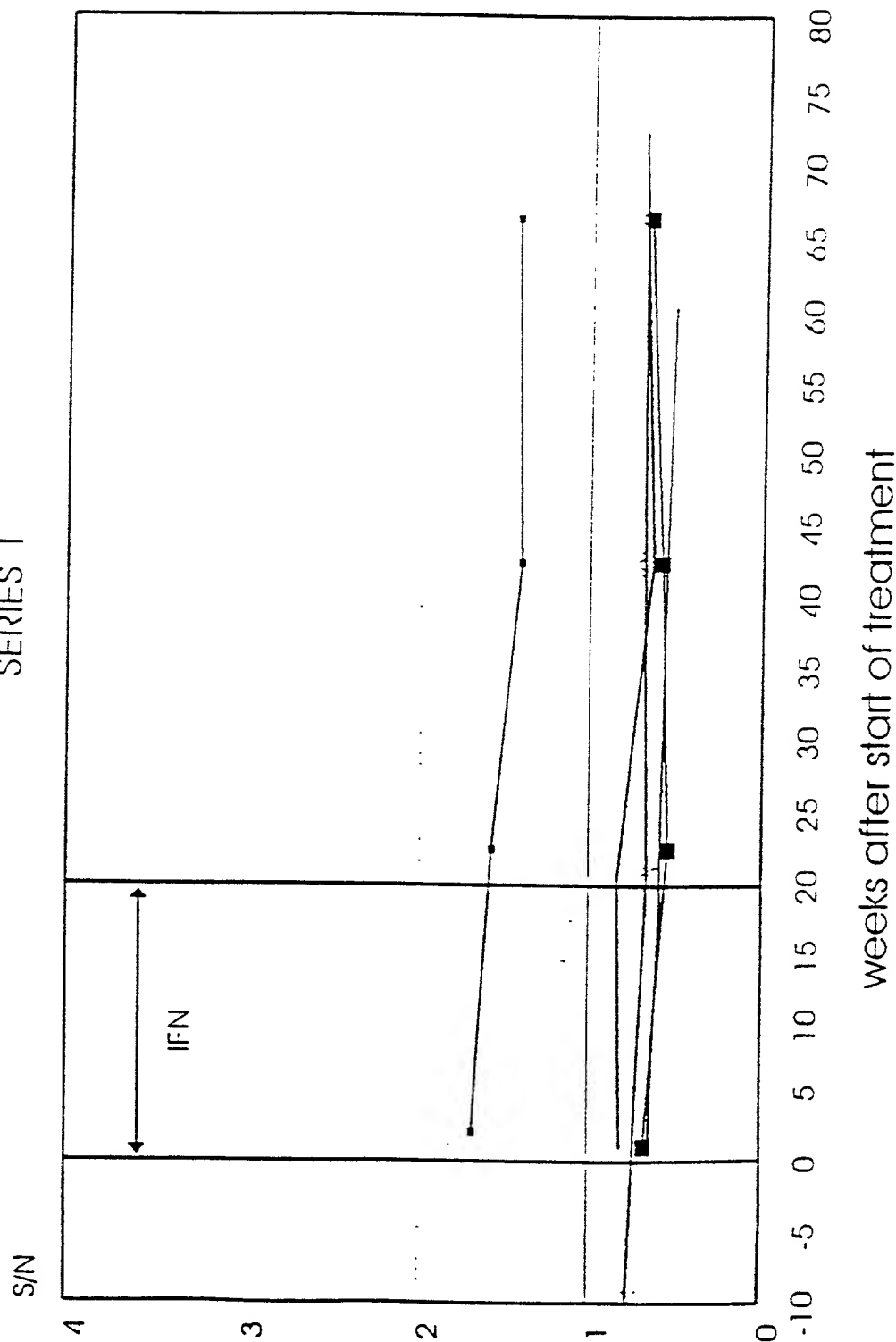
FIGURE 19

Competition of reactivity of anti-E2 Mabs with peptides

FIGURE 20

Human anti-E2 reactivity competed with peptides
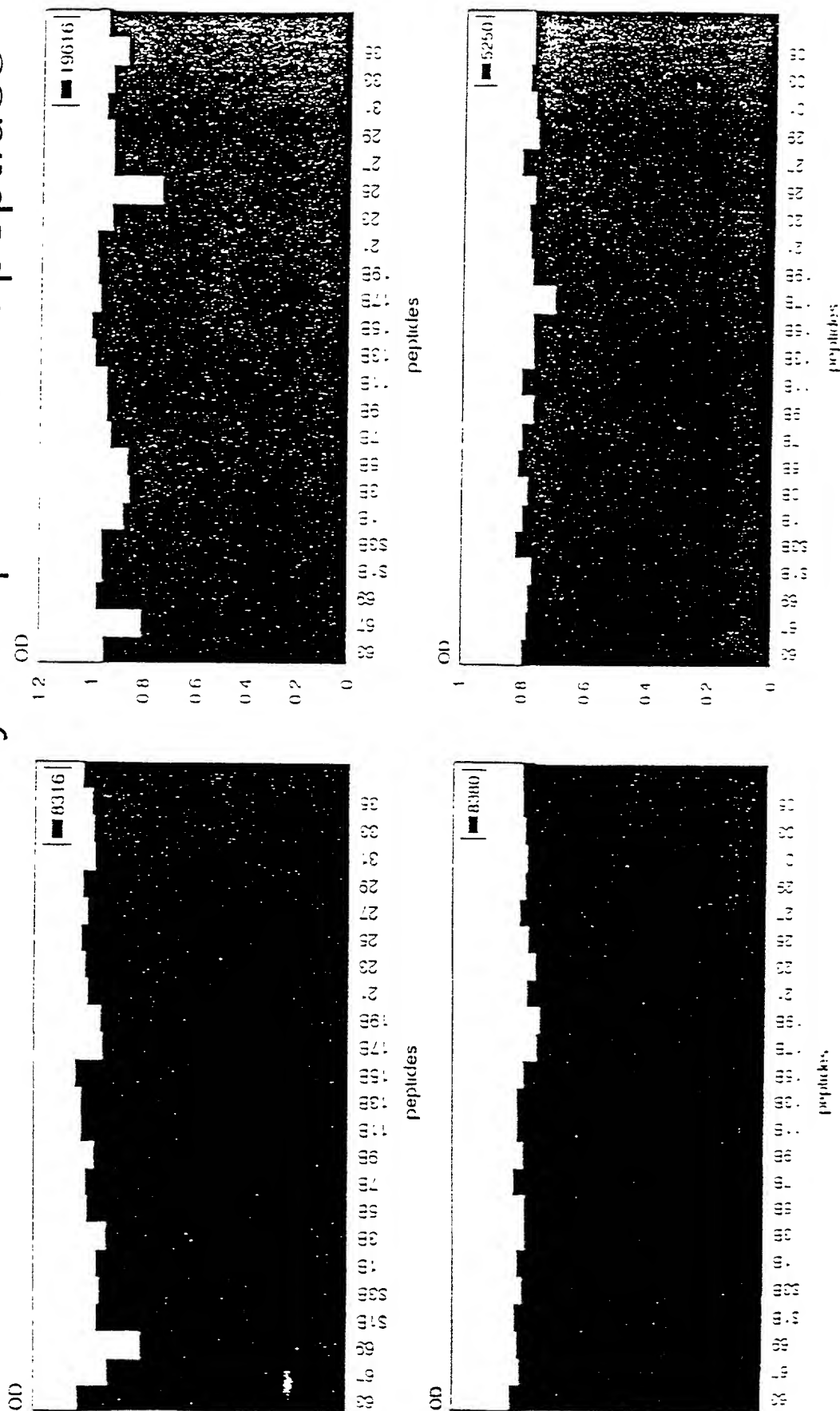
Figure 21

5' GGCATGCAAGCTTAATTAATT3' (SEQ ID NO 1)

3'ACGTCCGTACGTTCGAATTAATTAATCGA5' (SEQ ID NO 94)

5'CCGGGGAGGCCTGCACGTGATCGAGGGCAGACACCATCACCACCATCACTAATAGT

TAATTAACTGCA 3' (SEQ ID NO 2)

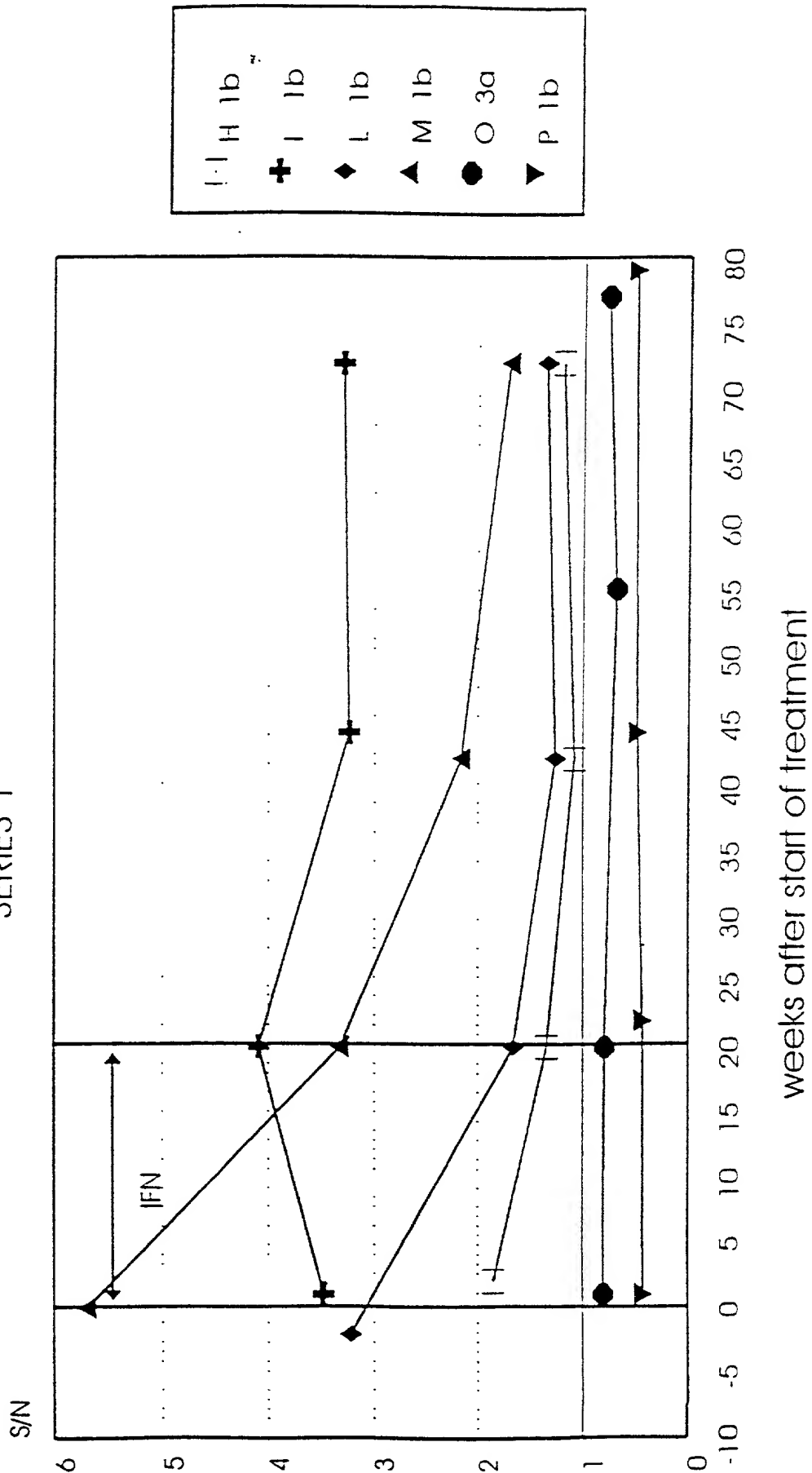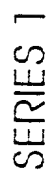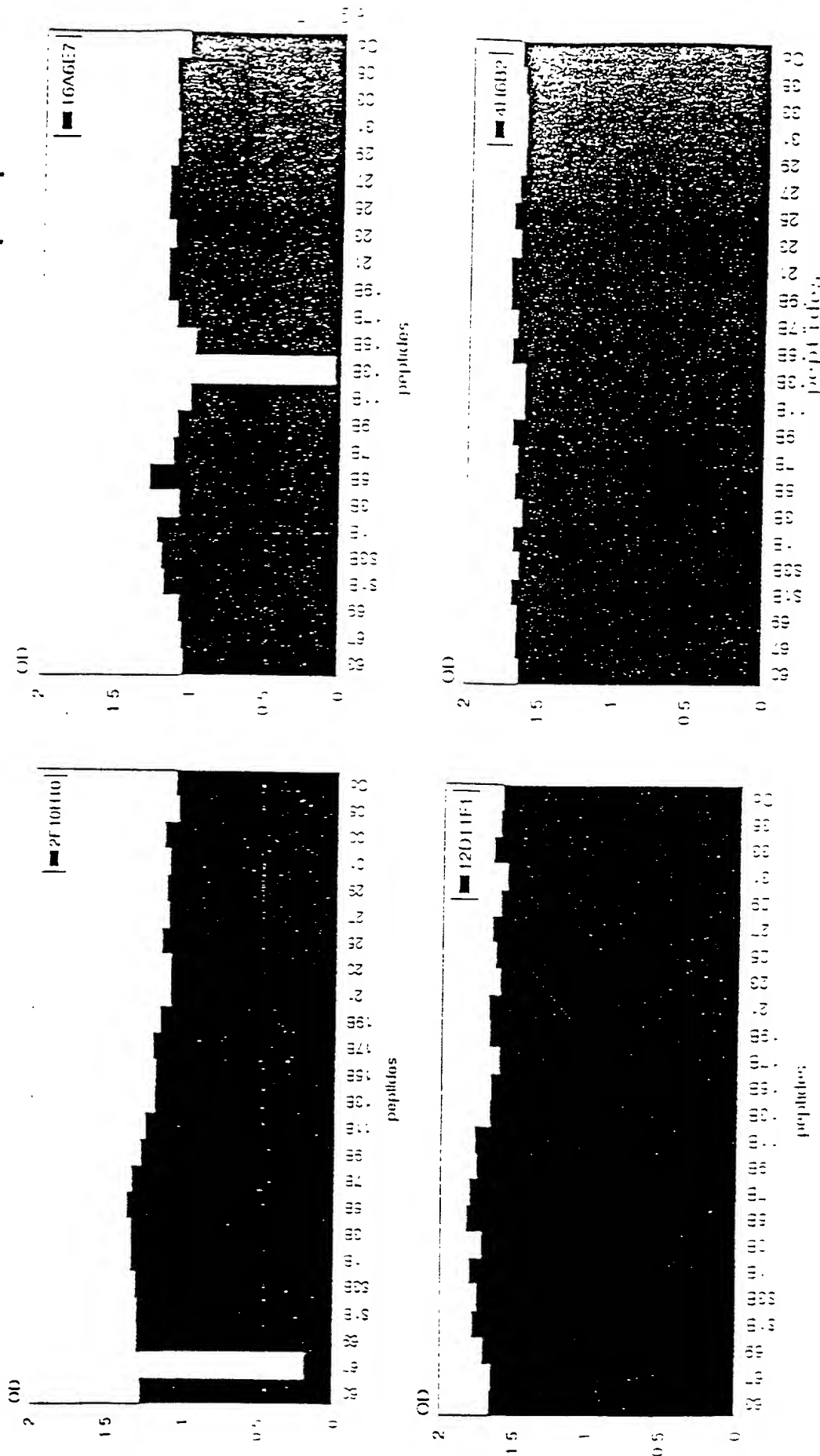3'CCTCCGGACGTGCACTAGCTCCCGTCTGTGGTAGTGGTGGTAGTGATTATCAATTAATTG

5' (SEQ ID NO 95)

SEQ ID NO 3 (HCCI9A)

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GCTTCCGCTTATGAGGTGCGCAACGTGTCCGGGATGTACCATGTCACGAACGACTGCT
CCAACTCAAGCATTGTGTATGAGGCAGCGGACATGATCATGCACACCCCCGGGTGCGT
GCCCTGCGTTCGGGAGAACAACTCTTCCCGCTGCTGGGTAGCGCTCACCCCCACGCTC
GCAGCTAGGAACGCCAGCGTCCCCACCACGACAATACGACGCCACGTCGATTTGCTCG
TTGGGGCGGCTGCTCTCTGTTCCGCTATGTACGTGGGGGATCTCTGCGGATCTGTCTTC
CTCGTCTCCCAGCTGTTCACCATCTCGCCTCGCCGGCATGAGACGGTGCAGGACTGCA
ATTGCTCAATCTATCCCGGCCACATAACAGGTCACCGTATGGCTTGGGATATGATGAT
GAACTGGTCGCCTACAACGGCCCTGGTGGTATCGCAGCTGCTCCGGATCCCACAAGCT
GTCGTGGACATGGTGGCGGGGGCCCATTGGGGAGTCCTGGCGGGCCTCGCCTACTATT
CCATGGTGGGGAACTGGGCTAAGGTTTTGATTGTGATGCTACTCTTTGCTCTCTAATAG

SEQ ID NO 5 (HCCI10A)

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TTCCGCTCGTCGGCGCCCCCCTAGGGGGCGCTGCCAGGGCCCTGGCGCATGGCGTCCG
GGTTCTGGAGGACGGCGTGAACTATGCAACAGGGAATTTGCCCGGTTGCTCTTTCTCT
ATCTTCCTCTTGGCTTTGCTGTCCTGTCTGACCGTTCCAGCTTCCGCTTATGAAGTGCG
CAACGTGTCCGGGATGTACCATGTCACGAACGACTGCTCCAACTCAAGCATTGTGTAT
GAGGCAGCGGACATGATCATGCACACCCCCGGGTGCGTGCCCTGCGTTCGGGAGAAC
AACTCTTCCCGCTGCTGGGTAGCGCTCACCCCCACGCTCGCAGCTAGGAACGCCAGCG
TCCCCACCACGACAATACGACGCCACGTCGATTTGCTCGTTGGGGCGGCTGCTTTCTG

TTCCGCTATGTACGTGGGGGACCTCTGCGGATCTGTCTTCCTCGTCTCCCAGCTGTTCA
CCATCTCGCCTCGCCGGCATGAGACGGTGCAGGACTGCAATTGCTCAATCTATCCCGG
CCACATAACGGGTCACCGTATGGCTTGGGATATGATGATGAACTGGTCGCCTACAACG
GCCCTGGTGGTATCGCAGCTGCTCCGGATCCCACAAGCTGTCGTGGACATGGTGGCGG
GGGCCCATTGGGGAGTCCTGGCGGGTCTCGCCTACTATTCCATGGTGGGGAACTGGGC
TAAGGTTTTGATTGTGATGCTACTCTTTGCTCCCTAATAG

SEQ ID NO 7 (HCCI11A)

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GGTTCTGGAAGACGGCGTGAACTATGCAACAGGGAATTTGCCTGGTTGCTCTTTCTCTA
TCTTCCTCTTGGCTTTACTGTCCTGTCTGACCATTCCAGCTTCCGCTTATGAGGTGCGC
AACGTGTCCGGGATGTACCATGTCACGAACGACTGCTCCAACTCAAGCATTGTGTATG
AGGCAGCGGACATGATCATGCACACCCCCGGGTGCGTGCCCTGCGTTCGGGAGAACA
ACTCTTCCCGCTGCTGGGTAGCGCTCACCCCCACGCTCGCAGCTAGGAACGCCAGCGT
CCCCACTACGACAATACGACGCCACGTCGATTTGCTCGTTGGGGCGGCTGCTTTCTGTT
CCGCTATGTACGTGGGGGATCTCTGCGGATCTGTCTTCCTCGTCTCCCAGCTGTTCACC
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ACATAACAGGTCACCGTATGGCTTGGGATATGATGATGAACTGGTAATAG

SEQ ID NO 9 (HCCI12A)

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GCTTCCGCTTATGAAGTGCGCAACGTGTCCGGGGTGTACCATGTCACGAACGACTGCT
CCAACTCAAGCATAGTGTATGAGGCAGCGGACATGATCATGCACACCCCCGGGTGCGT
GCCCTGCGTTCGGGAGGGCAACTCCTCCCGTTGCTGGGTGGCGCTCACTCCCACGCTC
GCGGCCAGGAACGCCAGCGTCCCCACAACGACAATACGACGCCACGTCGATTTGCTC
GTTGGGGCTGCTGCTTTCTGTTCCGCTATGTACGTGGGGGATCTCTGCGGATCTGTTTT
CCTTGTTTCCCAGCTGTTCACCTTCTCACCTCGCCGGCATCAAACAGTACAGGACTGCA
ACTGCTCAATCTATCCCGGCCATGTATCAGGTCACCGCATGGCTTGGGATATGATGAT
GAACTGGTCCTAATAG

SEQ ID NO 11 (HCCI13A)

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GCTTCCGCTTATGAAGTGCGCAACGTGTCCGGGGTGTACCATGTCACGAACGACTGCT
CCAACTCAAGCATAGTGTATGAGGCAGCGGACATGATCATGCACACCCCCGGGTGCGT

GCCCTGCGTTCGGGAGGGCAACTCCTCCCGTTGCTGGGTGGCGCTCACTCCCACGCTC
GCGGCCAGGAACGCCAGCGTCCCCACAACGACAATACGACGCCACGTCGATTTGCTC
GTTGGGGCTGCTGCTTTCTGTTCCGCTATGTACGTGGGGGATCTCTGCGGATCTGTTTT
CCTTGTTTCCCAGCTGTTCACCTTCTCACCTCGCCGGCATCAAACAGTACAGGACTGCA
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GAACTGGTAATAG

SEQ ID NO 13 (HCCI17A)
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GGTTCTGGAAGACGGCGTGAACTATGCAACAGGGAATTTGCCTGGTTGCTCTTTCTCTA
TCTTCCTCTTGGCTTTACTGTCCTGTCTAACCATTCCAGCTTCCGCTTACGAGGTGCGC
AACGTGTCCGGGATGTACCATGTCACGAACGACTGCTCCAACTCAAGCATTGTGTATG
AGGCAGCGGACATGATCATGCACACCCCCGGGTGCGTGCCCTGCGTTCGGGAGAACA
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CCCCACTACAACAATACGACGCCACGTCGATTTGCTCGTTGGGGCGGCTGCTTTCTGTT
CCGCTATGTACGTGGGGGATCTCTGCGGATCTGTCTTCCTCGTCTCCCAGCTGTTCACC
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ACATAACGGGTCACCGTATGGCTTGGGATATGATGATGAACTGGTACTAATAG

SEQ ID NO 15 (HCPr51)
ATGCCCGGTTGCTCTTTCTCTATCTT

SEQ ID NO 16 (HCPr52)
ATGTTGGGTAAGGTCATCGATACCCT

SEQ ID NO 17 (HCPr53)
CTATTAGGACCAGTTCATCATCATATCCCA

SEQ ID NO 18 (HCPr54)
CTATTACCAGTTCATCATCATATCCCA

SEQ ID NO 19 (HCPr107)
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SEQ ID NO 20 (HCPr108)

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SEQ ID NO 21 (HCCI37)

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GGTTCTGGAGGACGGCGTGAACTATGCAACAGGGAATTTGCCCGGTTGCTCTTTCTCT
ATCTTCCTCTTGGCTTTGCTGTCCTGTCTGACCGTTCCAGCTTCCGCTTATGAAGTGCG
CAACGTGTCCGGGATGTACCATGTCACGAACGACTGCTCCAACTCAAGCATTGTGTAT
GAGGCAGCGGACATGATCATGCACACCCCCGGGTGCGTGCCCTGCGTTCGGGAGAAC
AACTCTTCCCGCTGCTGGGTAGCGCTCACCCCCACGCTCGCAGCTAGGAACGCCAGCG
TCCCCACCACGACAATACGACGCCACGTCGATTCCCAGCTGTTCACCATCTCGCCTCG
CCGGCATGAGACGGTGCAGGACTGCAATTGCTCAATCTATCCCGGCCACATAACGGGT
CACCGTATGGCTTGGGATATGATGATGAACTGGTCGCCTACAACGGCCCTGGTGGTAT
CGCAGCTGCTCCGGATCCCACAAGCTGTCGTGGACATGGTGGCGGGGGCCCATTGGGG
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SEQ ID NO 23 (HCCI38)

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ATCTTCCTCTTGGCTTTGCTGTCCTGTCTGACCGTTCCAGCTTCCGCTTATGAAGTGCG
CAACGTGTCCGGGATGTACCATGTCACGAACGACTGCTCCAACTCAAGCATTGTGTAT
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CACCGTATGGCTTGGGATATGATGATGAACTGGTAA
TAG

SEQ ID NO 25 (HCCI39)

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ATCTTCCTCTTGGCTTTGCTGTCCTGTCTGACCGTTCCAGCTTCCGCTTATGAAGTGCG
CAACGTGTCCGGGATGTACCATGTCACGAACGACTGCTCCAACTCAAGCATTGTGTAT
GAGGCAGCGGACATGATCATGCACACCCCGGGTGCGTGCCCTGCGTTCGGGAGAAC
AACTCTTCCCGCTGCTGGGTAGCGCTCACCCCCACGCTCGCAGCTAGGAACGCCAGCG
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SEQ ID NO 27 (HCCl40)

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ATCTTCCTCTTGGCTTTGCTGTCCTGTCTGACCGTTCCAGCTTCCGCTTATGAAGTGCG
CAACGTGTCCGGGATGTACCATGTCACGAACGACTGCTCCAACTCAAGCATTGTGTAT
GAGGCAGCGGACATGATCATGCACACCCCGGGTGCGTGCCCTGCGTTCGGGAGAAC
AACTCTTCCCGCTGCTGGGTAGCGCTCACCCCCACGCTCGCAGCTAGGAACGCCAGCG
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CCGGCATGAGACGGTGCAGGACTGCAATTGCTCAATCTATCCCGGCCACATAACGGGT
CACCGTATGGCTTGGGATATGATGATGAACTGGTCGCCTACAACGGCCCTGGTGGTAT
CGCAGCTGCTCCGGATCGTGATCGAGGGCAGACACCATCACCACCATCACTAATAG

SEQ ID NO 29 (HCCl62)

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TCCTTCTCGCTCTGTTCTCTTGCTTAATTCATCCAGCAGCTAGTCTAGAGTGGCGGAAT
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SEQ ID NO 31 (HCCI63)

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TACCGGCCACCGGATGGCATGGGATATGATGATGAACTGGTAATAG

SEQ ID NO 33 (HCPr109)

TGGGATATGATGATGAACTGGTC

SEQ ID NO 34 (HCPr72)

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SEQ ID NO 35 (HCCL22A)

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TGTACATGGATGAATGGCACTGGGTTCACCAAGACGTGTGGGGGCCCCCCGTGCAACA
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ACTCTTTCCTTCCTTGTGTTCTTCTGTGCTGCCTGGTACATCAAGGGCAGGCTGGTCCC
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SEQ ID NO 37 (HCCI41)

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TTGCCGGCGTCGACGGGCATACCCGCGTGTCAGGAGGGGCAGCAGCCTCCGATACCA
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TCGGGGGGGCCGGCAACAACACCTTGACCTGCCCCACTGACTGTTTTCGGAAGCACCC
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CATTACCCATATAGGCTCTGGCACTACCCCTGCACTGTCAACTTCACCATCTTCAAGGT
TAGGATGTACGTGGGGGGCGTGGAGCACAGGTTCGAAGCCGCATGCAATTGGACTCG
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SEQ ID NO 39 (HCCI42)

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AGGGTTCTTTGCCGCACTATTCTACAAACACAAATTCAACTCGTCTGGATGCCCAGAG
CGCTTGGCCAGCTGTCGCTCCATCGACAAGTTCGCTCAGGGGTGGGGTCCCCTCACTT
ACACTGAGCCTAACAGCTCGGACCAGAGGCCCTACTGCTGGCACTACGCGCCTCGACC
GTGTGGTATTGTACCCGCGTCTCAGGTGTGCGGTCCAGTGTATTGCTTCACCCCGAGCC
CTGTTGTGGTGGGGACGACCGATCGGTTTGGTGTCCCCACGTATAACTGGGGGGCGAA
CGACTCGGATGTGCTGATTCTCAACAACACGCGGCCGCCGCGAGGCAACTGGTTCGGC
TGTACATGGATGAATGGCACTGGGTTCACCAAGACGTGTGGGGGCCCCCGTGCAACA
TCGGGGGGGCCGGCAACAACACCTTGACCTGCCCCACTGACTGTTTTCGGAAGCACCC
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CATTACCCATATAGGCTCTGGCACTACCCCTGCACTGTCAACTTCACCATCTTCAAGGT
TAGGATGTACGTGGGGGGCGTGGAGCACAGGTTCGAAGCCGCATGCAATTGGACTCG
AGGAGAGCGTTGTGACTTGGAGGACAGGGATAGATCAGAGCTTAGCCCGCTGCTGCTG
TCTACAACAGGTGATCGAGGGCAGACACCATCACCACCATCACTAATAG

SEQ ID NO 41 (HCCI43)

ATGGTGGGGAACTGGGCTAAGGTTTTGGTTGTGATGCTACTCTTTGCCGGCGTCGACG
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CTTTAGCCCCGGGTCGGCTCAGAAAATCCAGCTCGTAAACACCAACGGCAGTTGGCAC
ATCAACAGGACTGCCCTGAACTGCAACGACTCCCTCCAAACAGGGTTCTTTGCCGCAC
TATTCTACAAACACAAATTCAACTCGTCTGGATGCCCAGAGCGCTTGGCCAGCTGTCG
CTCCATCGACAAGTTCGCTCAGGGGTGGGGTCCCCTCACTTACACTGAGCCTAACAGC
TCGGACCAGAGGCCCTACTGCTGGCACTACGCGCCTCGACCGTGTGGTATTGTACCCG
CGTCTCAGGTGTGCGGTCCAGTGTATTGCTTCACCCCGAGCCCTGTTGTGGTGGGGAC
GACCGATCGGTTTGGTGTCCCCACGTATAACTGGGGGGCGAACGACTCGGATGTGCTG
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CAGATGCGGTTCTGGGCCCTGGCTGACACCTAGGTGTATGGTTCATTACCCATATAGG
CTCTGGCACTACCCCTGCACTGTCAACTTCACCATCTTCAAGGTTAGGATGTACGTGGG
GGGCGTGGAGCACAGGTTCGAAGCCGCATGCAATTGGACTCGAGGAGAGCGTTGTGA
CTTGGAGGACAGGGATAGATCAGAGCTTAGCCCGCTGCTGCTGTCTACAACAGAGTGG
CAGAGCTTAATTAATTAG

SEQ ID NO 43 (HCCI44)

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GGCATACCCGCGTGTCAGGAGGGGCAGCAGCCTCCGATACCAGGGGCCTTGTGTCCCT
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TATTCTACAAACACAAATTCAACTCGTCTGGATGCCCAGAGCGCTTGGCCAGCTGTCG
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CGTCTCAGGTGTGCGGTCCAGTGTATTGCTTCACCCCGAGCCCTGTTGTGGTGGGGAC
GACCGATCGGTTTGGTGTCCCCACGTATAACTGGGGGGCGAACGACTCGGATGTGCTG
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GCACTGGGTTCACCAAGACGTGTGGGGGCCCCCCGTGCAACATCGGGGGGGCCGGCA
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CAGATGCGGTTCTGGGCCCTGGCTGACACCTAGGTGTATGGTTCATTACCCATATAGG
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SEQ ID NO 45 (HCCL64)

ATGGTGGCGGGGGCCCATTGGGGAGTCCTGGCGGGCCTCGCCTACTATTCCATGGTGG
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CCGCGTGTCAGGAGGGGCAGCAGCCTCCGATACCAGGGGCCTTGTGTCCCTCTTTAGC
CCCGGGTCGGCTCAGAAAATCCAGCTCGTAAACACCAACGGCAGTTGGCACATCAAC
AGGACTGCCCTGAACTGCAACGACTCCCTCCAAACAGGGTTCTTTGCCGCACTATTCT
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CGACAAGTTCGCTCAGGGGTGGGGTCCCCTCACTTACACTGAGCCTAACAGCTCGGAC
CAGAGGCCCTACTGCTGGCACTACGCGCCTCGACCGTGTGGTATTGTACCCGCGTCTC
AGGTGTGCGGTCCAGTGTATTGCTTCACCCCGAGCCCTGTTGTGGTGGGGACGACCGA
TCGGTTTGGTGTCCCCACGTATAACTGGGGGGCGAACGACTCGGATGTGCTGATTCTC
AACAACACGCGGCCGCCGCGAGGCAACTGGTTCGGCTGTACATGGATGAATGGCACT
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GAACATCGTGGACGTGCAATACCTGTACGGTGTAGGGTCGGCGGTTGTCTCCCTTGTC
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CTGCTTATGGATGATGCTGCTGATAGCTCAAGCTGAGGCCGCCTTAGAGAACCTGGTG
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CTGTGCTGCCTGGTACATCAAGGGCAGGCTGGTCCCTGGTGCGGCATACGCCTTCTAT
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SEQ ID NO 47 (HCCI65)

AATTTGGGTAAGGTCATCGATACCCTTACATGCGGCTTCGCCGACCTCGTGGGGTACA
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ATCTTCCTCTTGGCTTTGCTGTCCTGTCTGACCGTTCCAGCTTCCGCTTATGAAGTGCG
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TCCCCACCACGACAATACGACGCCACGTCGATTTGCTCGTTGGGGCGGCTGCTTTCTG
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CTGTCAACTTCACCATCTTCAAGGTTAGGATGTACGTGGGGGGCGTGGAGCACAGGTT

CGAAGCCGCATGCAATTGGACTCGAGGAGAGCGTTGTGACTTGGAGGACAGGGATAG

ATCAGAGCTTAGCCCGCTGCTGCTGTCTACAACAGAGTGGCAGATACTGCCCTGTTCC

TTCACCACCCTGCCGGCCCTATCCACCGGCCTGATCCACCTCCATCAGAACATCGTGG

ACGTGCAATACCTGTACGGTGTAGGGTCGGCGGTTGTCTCCCTTGTCATCAAATGGGA

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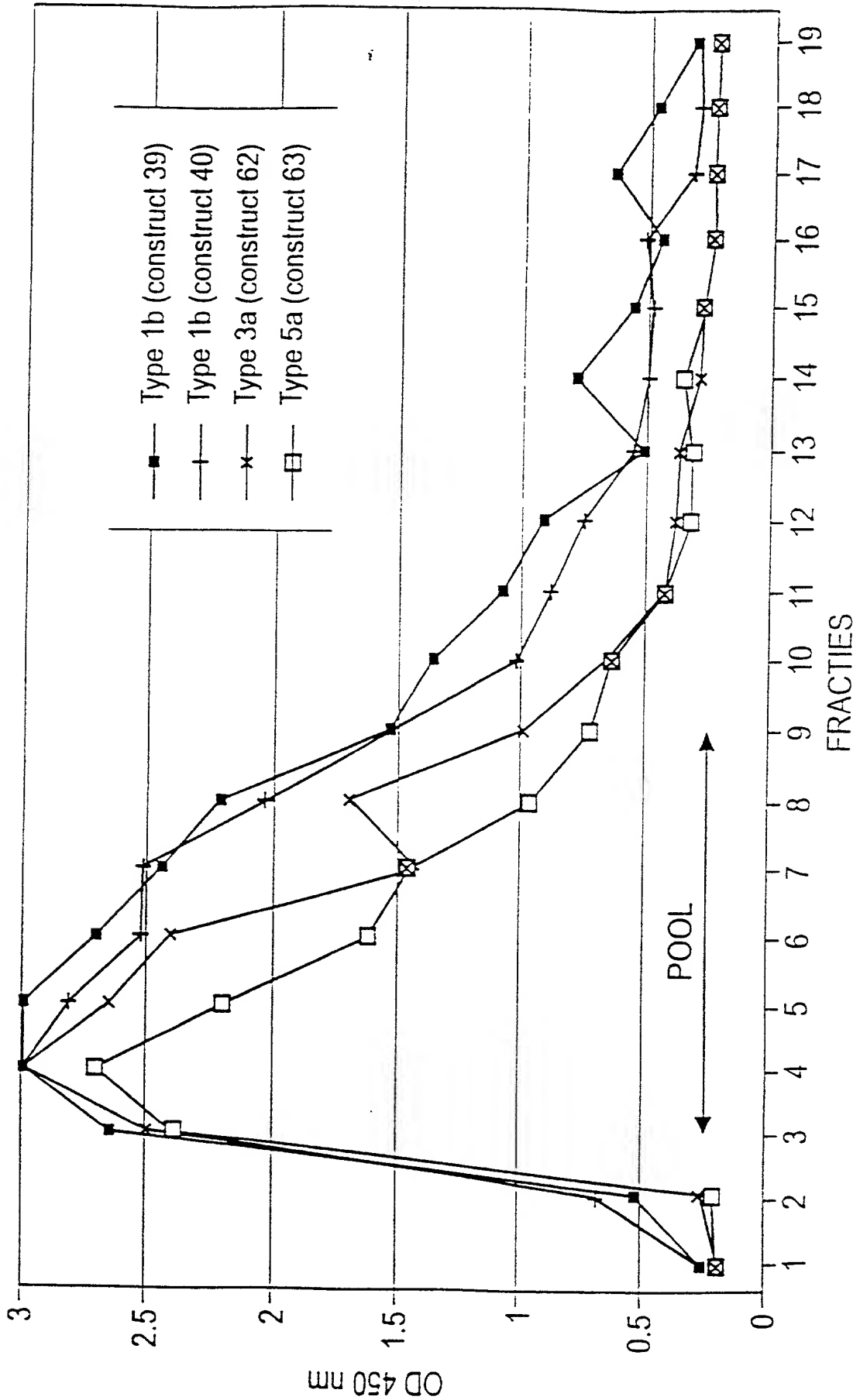
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SEQ ID NO 49 (HCCI66)

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GGTACATTCCGCTCGTCGGCGCCCCCTAGGGGGCGCTGCCAGGGCCCTGGCGCATGG

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GTGCGCAACGTGTCCGGGATGTACCATGTCACGAACGACTGCTCCAACTCAAGCATTG

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GAACAACTCTTCCCGCTGCTGGGTAGCGCTCACCCCCACGCTCGCAGCTAGGAACGCC

AGCGTCCCCACCACGACAATACGACGCCACGTCGATTTGCTCGTTGGGGCGGCTGCTT

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GTCAGGAGGGGCAGCAGCCTCCGATACCAGGGGCCTTGTGTCCCTCTTTAGCCCCGGG

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GCCCTGAACTGCAACGACTCCCTCCAAACAGGGTTCTTTGCCGCACTATTCTACAAAC

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GCGGTCCAGTGTATTGCTTCACCCCGAGCCCTGTTGTGGTGGGGACGACCGATCGGTT

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CCCTGCACTGTCAACTTCACCATCTTCAAGGTTAGGATGTACGTGGGGGGCGTGGAGC

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CTGTTCCTTCACCACCCTGCCGGCCCTATCCACCGGCCTGATCCACCTCCATCAGAAC

ATCGTGGACGTGCAATACCTGTACGGTGTAGGGTCGGCGGTTGTCTCCCTTGTCATCA

AATGGGAGTATGTCCTGTTGCTCTTCCTTCTCCTGGCAGACGCGCGCATCTGCGCCTGC

TTATGGATGATGCTGCTGATAGCTCAAGCTGAGGCCGCCTTAGAGAACCTGGTGGTCC

TCAATGCGGCGGCCGTGGCCGGGGCGCATGGCACTCTTTCCTTCCTTGTGTTCTTCTGT

GCTGCCTGGTACATCAAGGGCAGGCTGGTCCCTGGTGCGGCATACGCCTTCTATGGCG

TGTGGCCGCTGCTCCTGCTTCTGCTGGCCTTACCACCACGAGCTTATGCCTAGTAA

Figure 22

OD measured at 450 nm
construct

Fraction	volume	dilution	39 Type 1b	40 Type 1b	62 Type 3a	63 Type 3a
START	23 ml	1/20	2.517	1.954	1 426	1.142
FLOW THROUGH	23 ml	1/20	0.057	0.085	0.176	0.120
1	0.4 ml	1/200	0.102	0.051	0.048	0.050
2			0.396	0.550	0.090	0.067
3			2.627	2.603	2.481	2.372
4			3	2.967	3	2.694
5			3	2.810	2.640	2.154
6			2.694	2.499	1.359	1.561
7			2.408	2.481	0.347	1.390
8			2.176	1.970	1.624	0.865
9			1.461	1.422	0.887	0.604
10			1.286	0.926	0.543	0.519
11			0.981	0.781	0.294	0.294
12			0.812	0.650	0.249	0.199
13			0.373	0.432	0.239	0.209
14			0.653	0.371	0.145	0.184
15			0.441	0.348	0.151	0.151
16			0.321	0.374	0.098	0.106
17			0.525	0.186	0.099	0.108
18			0.351	0.171	0.083	0.090
19			0.192	0.164	0.084	0.087

Legend:
- Type 1b (construct 39)
- Type 1b (construct 40)
- Type 3a (construct 62)
- Type 5a (construct 63)

X-axis: FRACTIES (1–19)
Y-axis: OD 450 nm (0–3)

POOL

Figure 24

			OD measured at 450 nm construct			
Fraction	volume	dilution	39 Type 1b	40 Type 1b	62 Type 3a	63 Type 5a
20	250 µl	1/200	0 072	0.130	0 096	0 051
21			0.109	0.293	0.084	0.052
22			0.279	0.249	0 172	0 052
23			0.093	0 151	0.297	0 054
24			0 080	0 266	0 438	0.056
25			0.251	0 100	0 457	0 048
26			3	1 649	0 722	0 066
27			3	3	2 528	0 889
28			3	3	3	2.345
29			3	3	2 849	2.580
30			2.227	1 921	1 424	1.333
31			0 263	0.415	0.356	0.162
32			0.071	0.172	0 154	0.064
33			0.103	0 054	0.096	0.057
34			0.045	0 045	0 044	0 051
35			0 043	0.047	0 045	0.046
36			0.045	0.045	0 049	0.040
37			0 045	0.047	0.046	0 048
38			0 046	0.048	0.047	0 057
39			0 045	0.048	0 050	0.057
40			0 046	0.049	0 048	0 049

FIGURE 25

Figure 26

67KD —
45KD —

29KD —

18 KD —

14 KD —

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
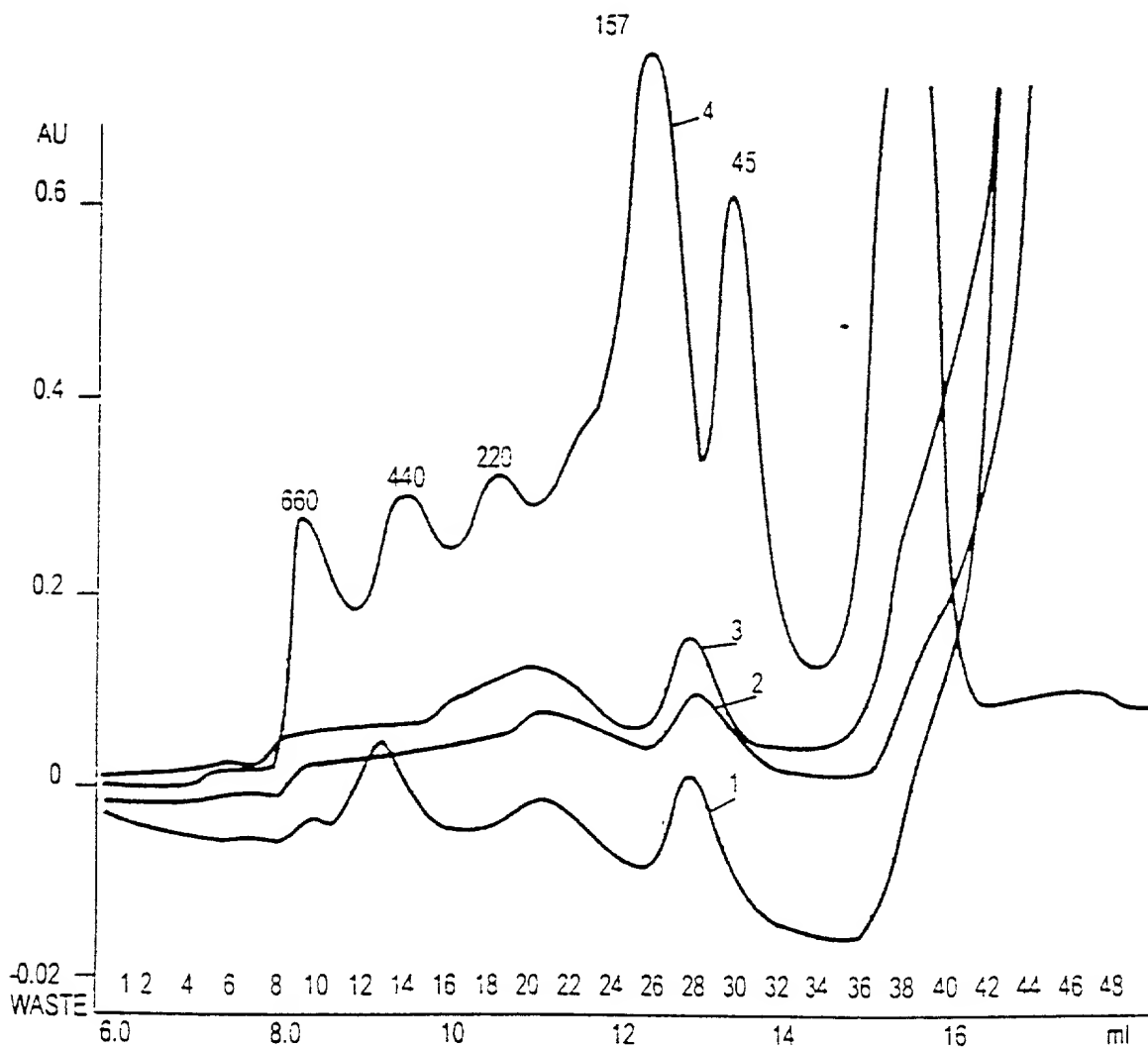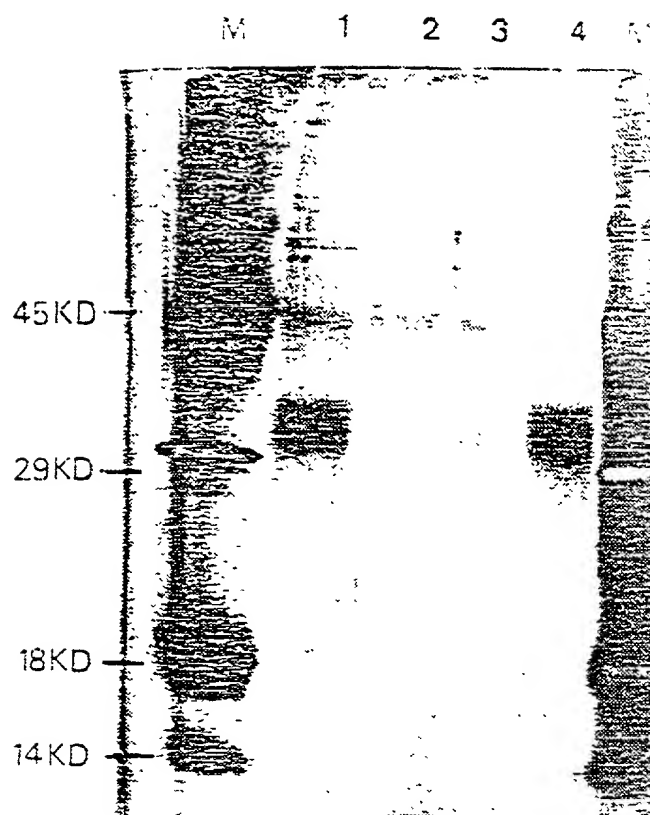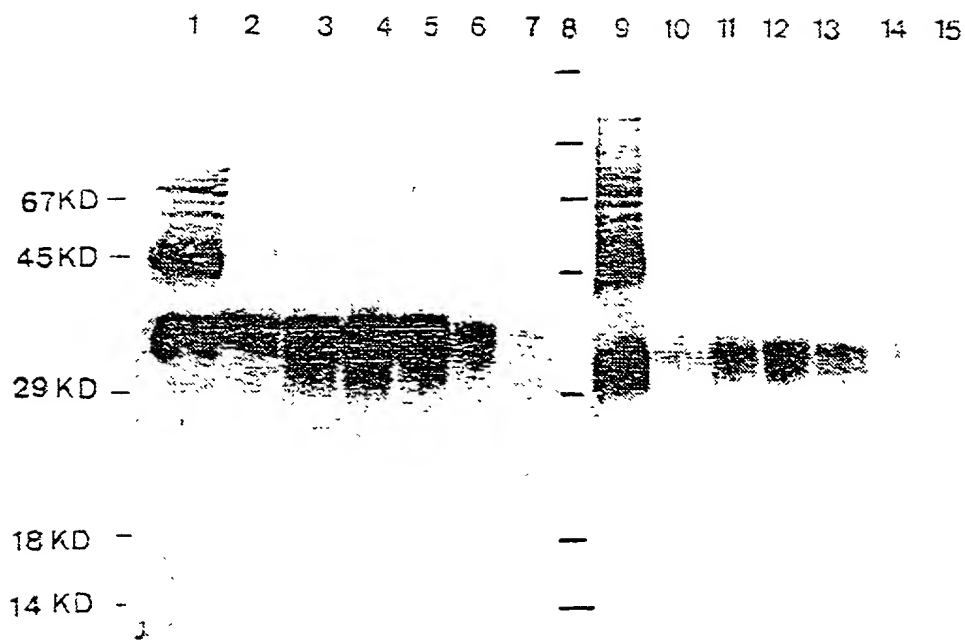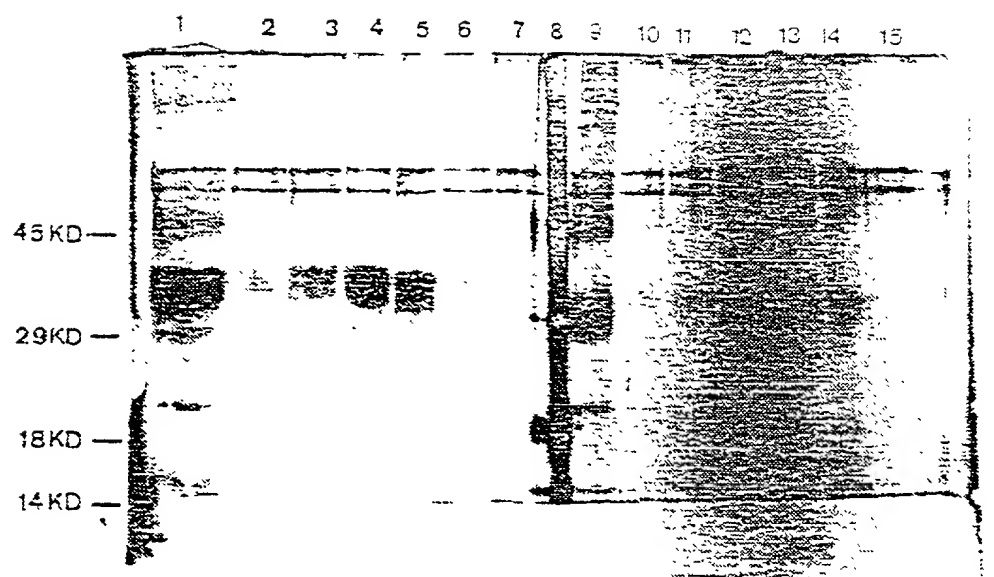
Figure 27

Figure 28
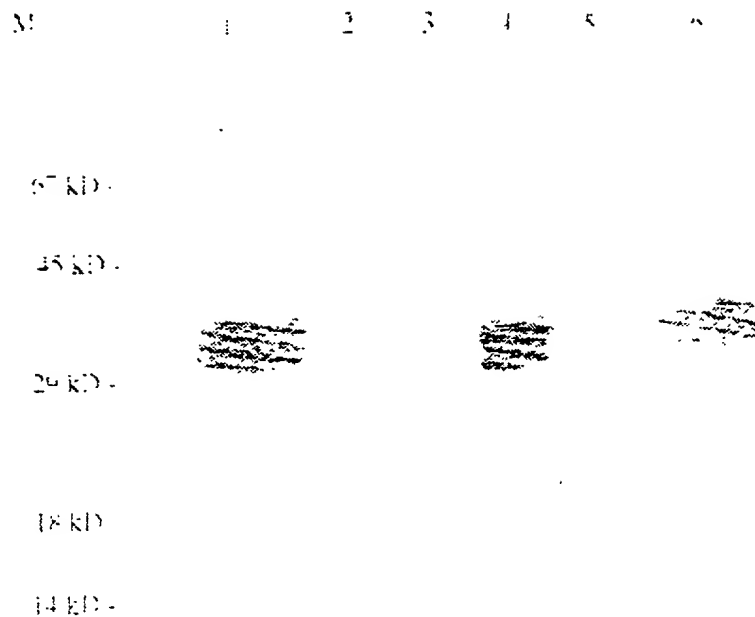
Lane 1: Crude Lysate
Lane 2: Flow through Lentil Chromatography
Lane 3: Wash with EMPIGEN Lentil Chromatography
Lane 4: Eluate Lentil Chromatography
Lane 5: Flow through during concentration lentil eluate
Lane 6: Pool of Flatter Size Exclusion Chromatography

Figure 29: Western Blot Analysis with anti-E1 mouse monoclonal 5E1A10
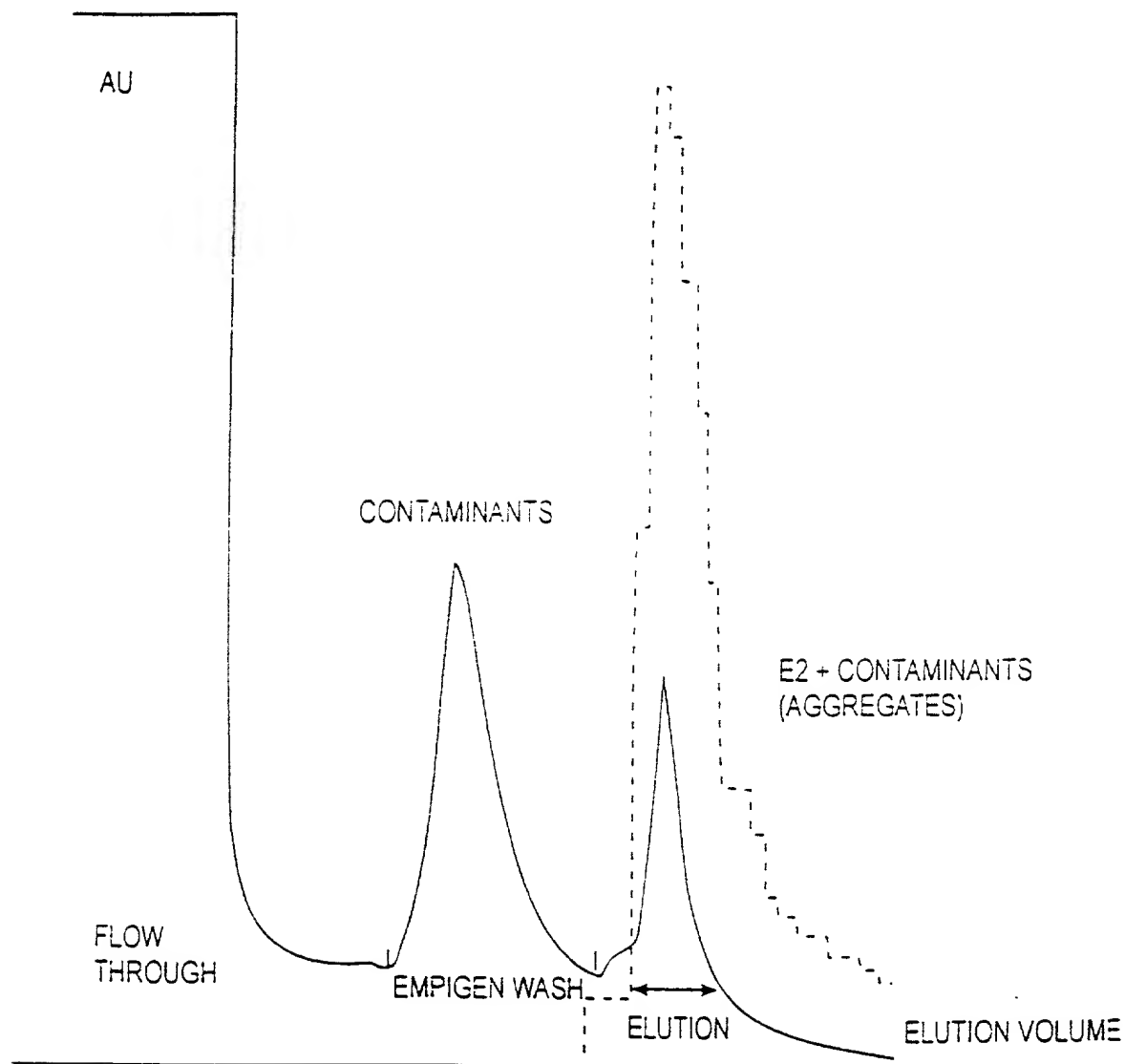
FIGURE 30

A: NON - REDUCED

E2 + CONTAMINANTS (AGGREGATES)

B : REDUCED

CONTAMINANTS

E2 + CONTAMINANTS

POOL
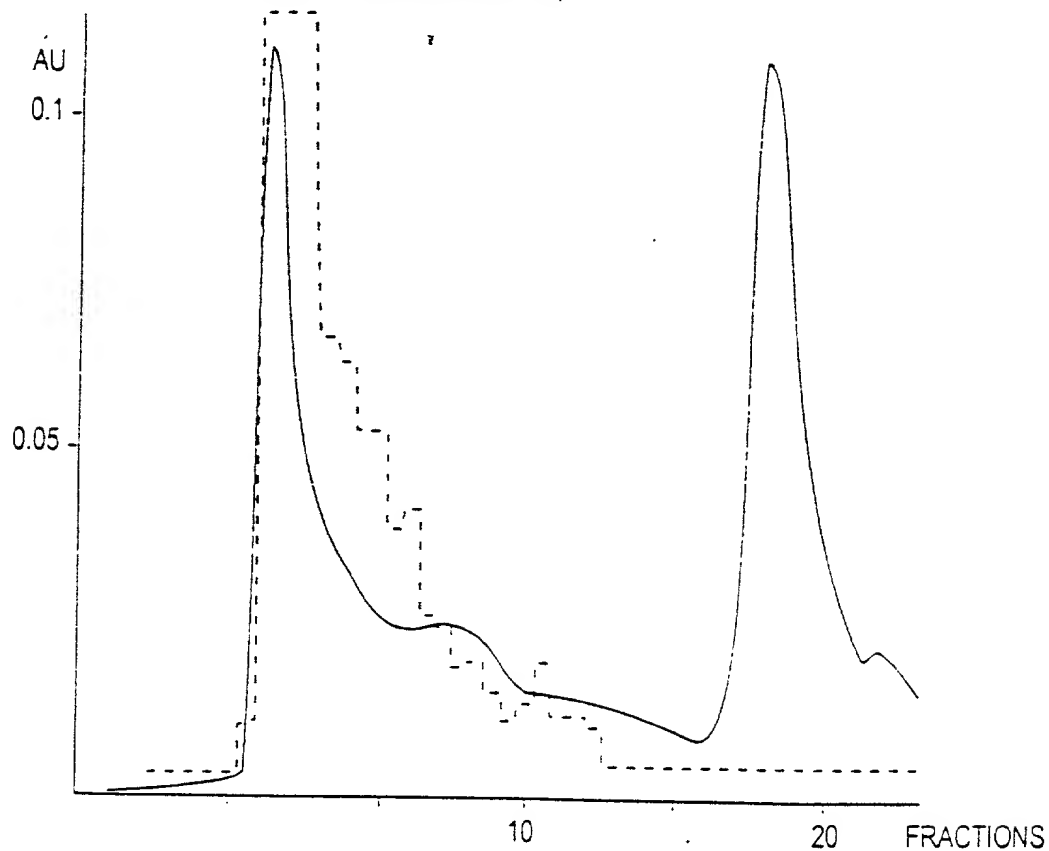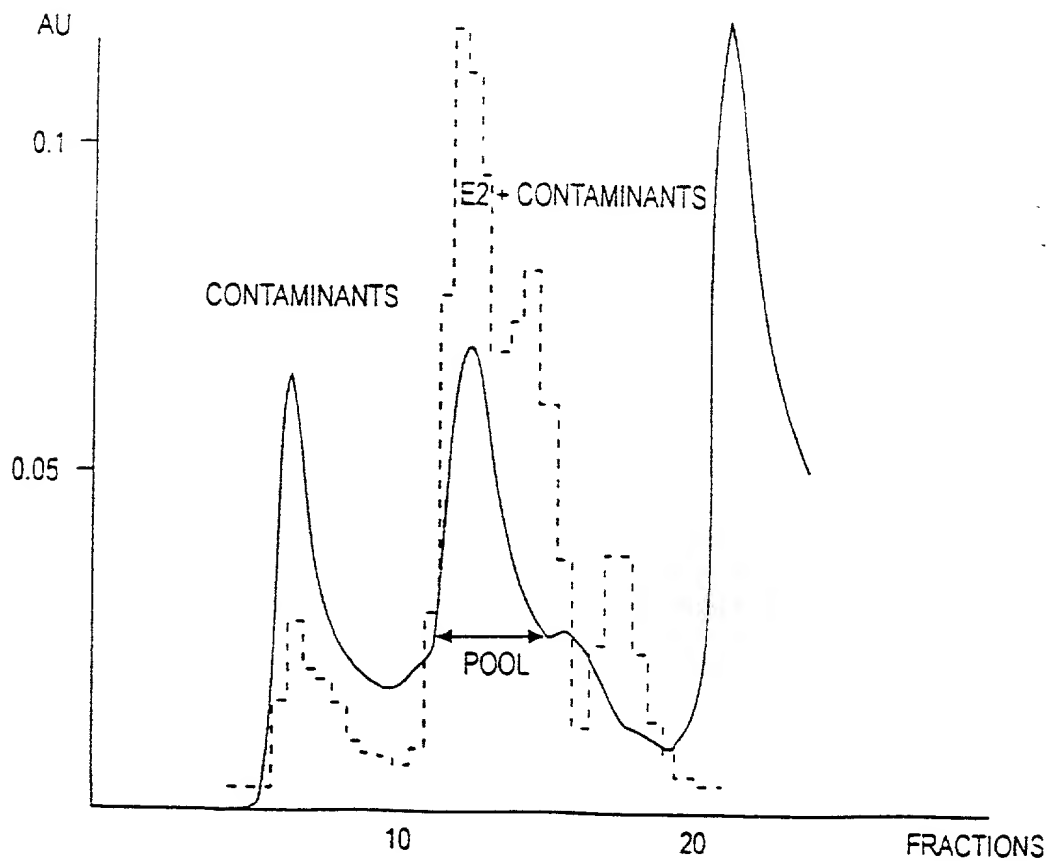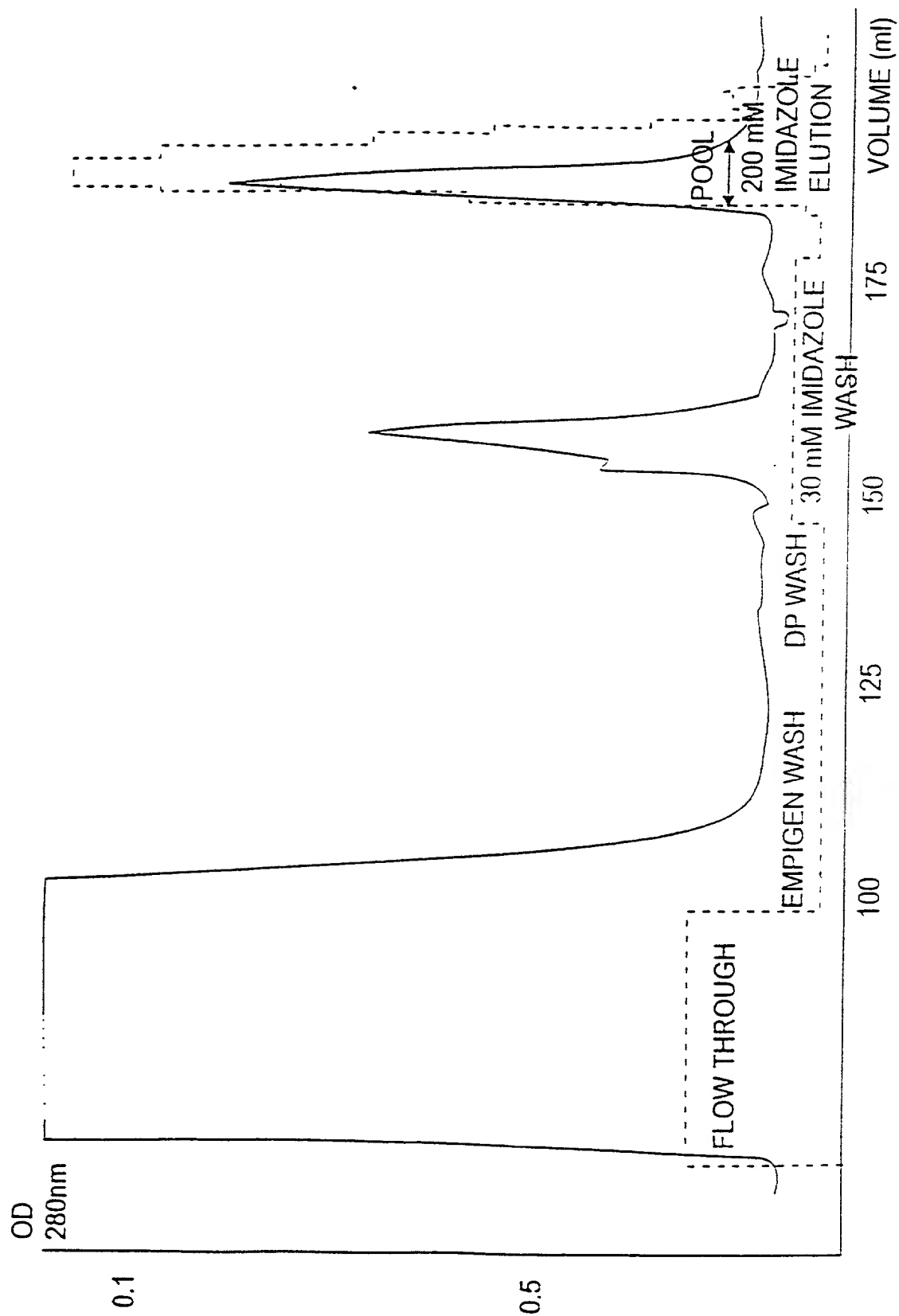
FIGURE 31

FIGURE 32

FIGURE 33:
SILVER STAIN OF PURIFIED E2

M   1   2

67 kD -

45 kD-

29 kD-

18 kD-

14 kD-
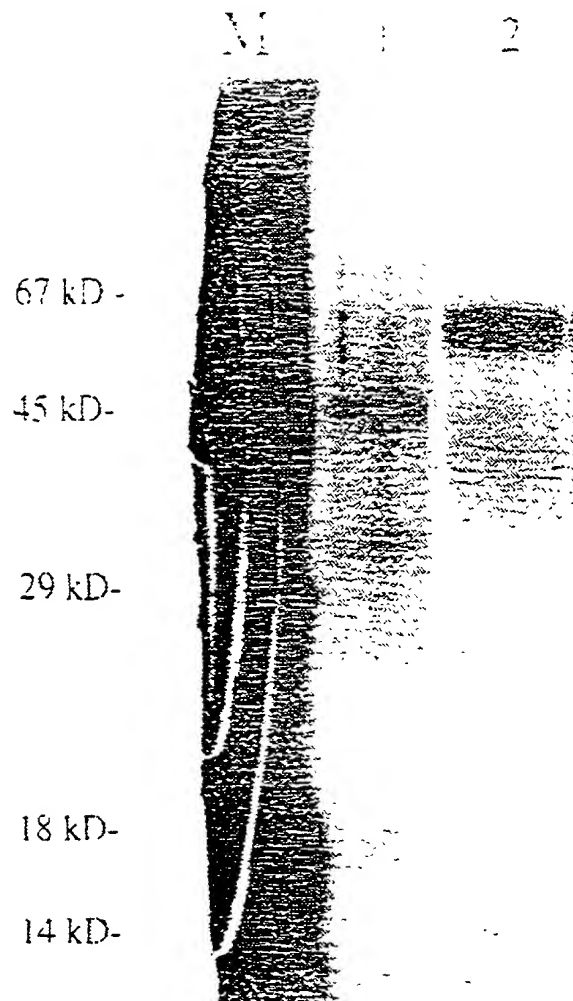
1. 30 mM IMIDAZOLE WASH Ni-IMAC
2. 0.5 µg E2

45 59 Figure 34
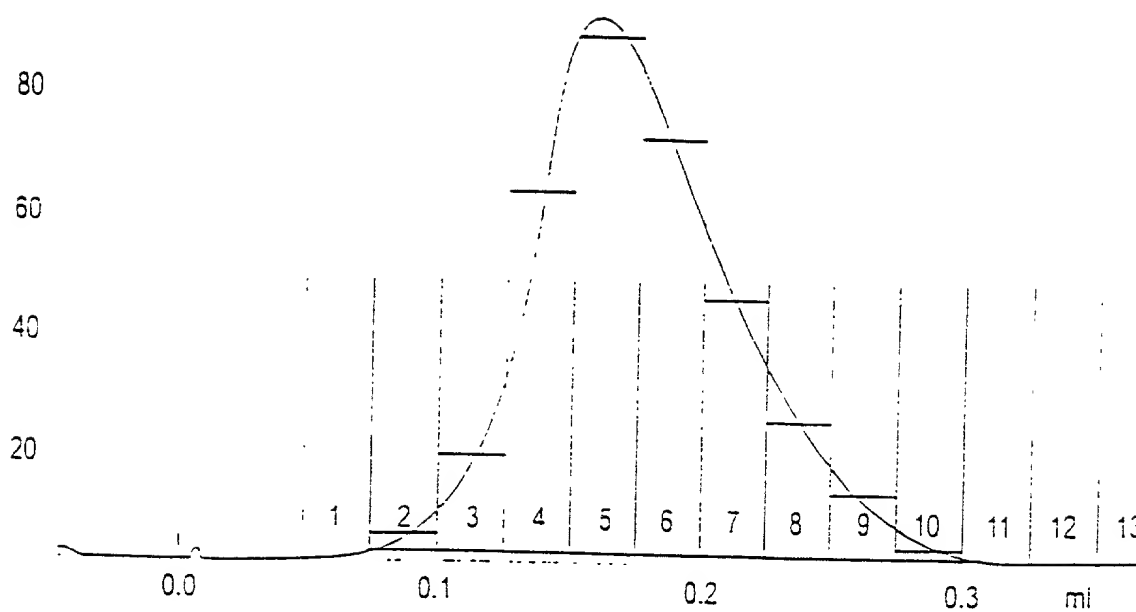
No.	Ret. (ml)	Peak start (ml)	Peak end (ml)	Dur (ml)	Area (ml*mAU)	Height (mAU)
1	-0.45	-0.46	-0.43	0.04	0.0976	4.579
2	1.55	0.75	3.26	2.51	796.4167	889.377
3	3.27	3.26	3.31	0.05	0.0067	0.224
4	3.33	3.32	3.33	0.02	0.0002	0.018

Total number of detected peaks = 4
Total Area above baseline = 0.796522 ml*AU
Total area in evaluated peaks = 0.796521 ml*AU
Ratio peak area / total area = 0.999999
Total peak duration = 2.613583 ml

FIGURE 35A

Core 1 Ab NR

Core 1 Ab LTR

Core 2 Ab NR

Core 2 Ab LTR

E2HVR Ab NR

E2HVR Ab LTR

NS3 Ab NR

NS3 Ab LTR
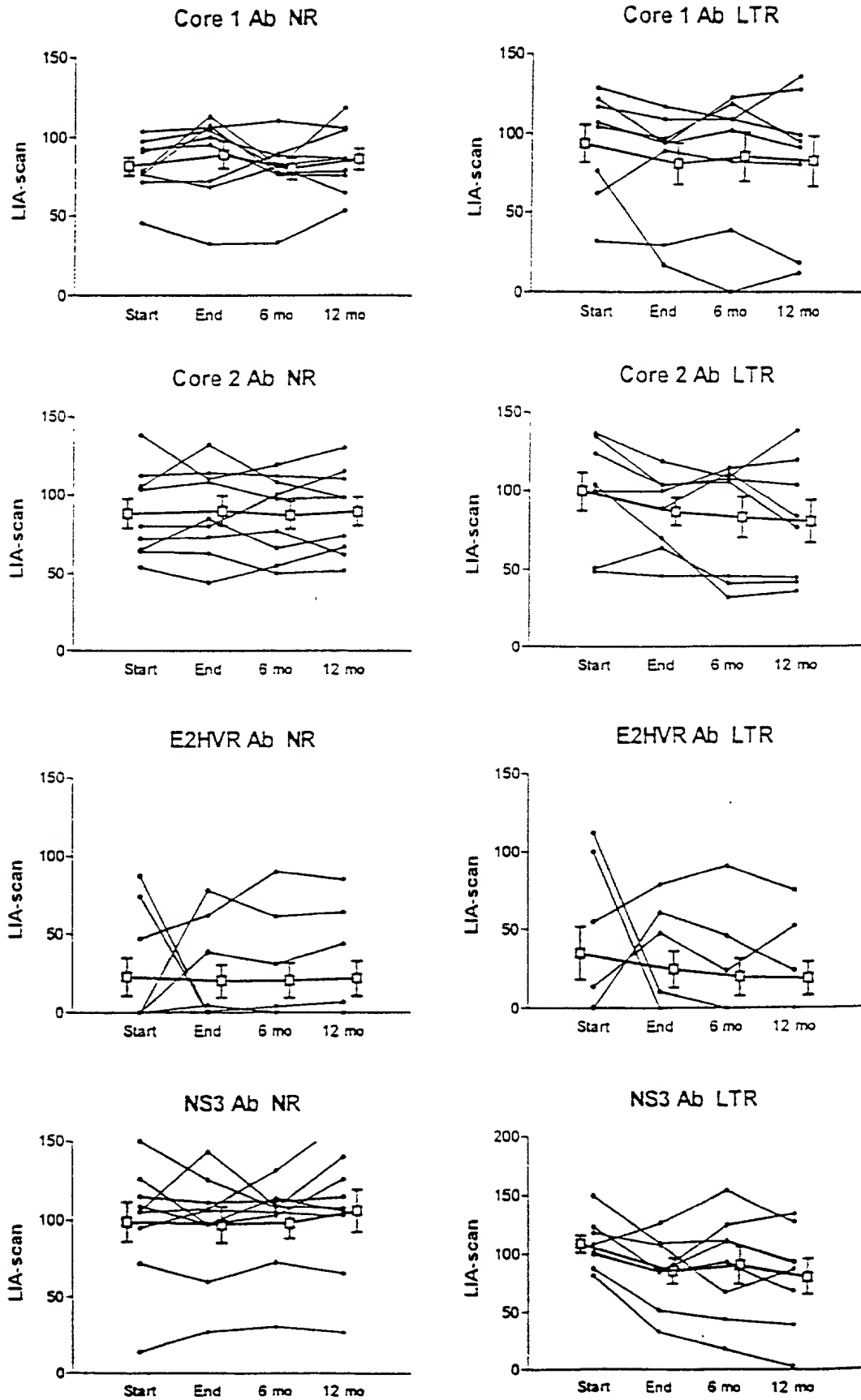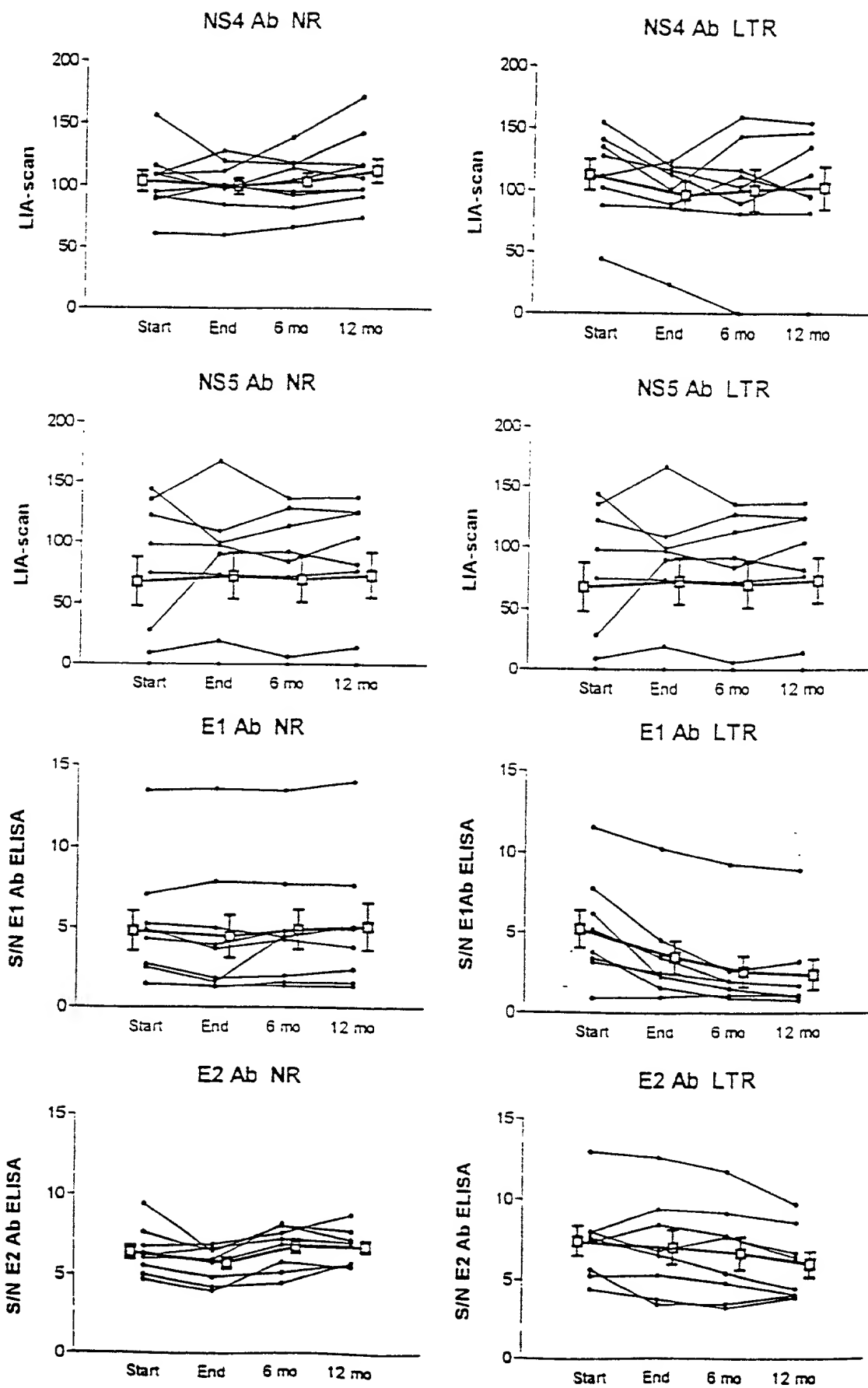
FIGURE 35B

Figure 36

E1 Ab
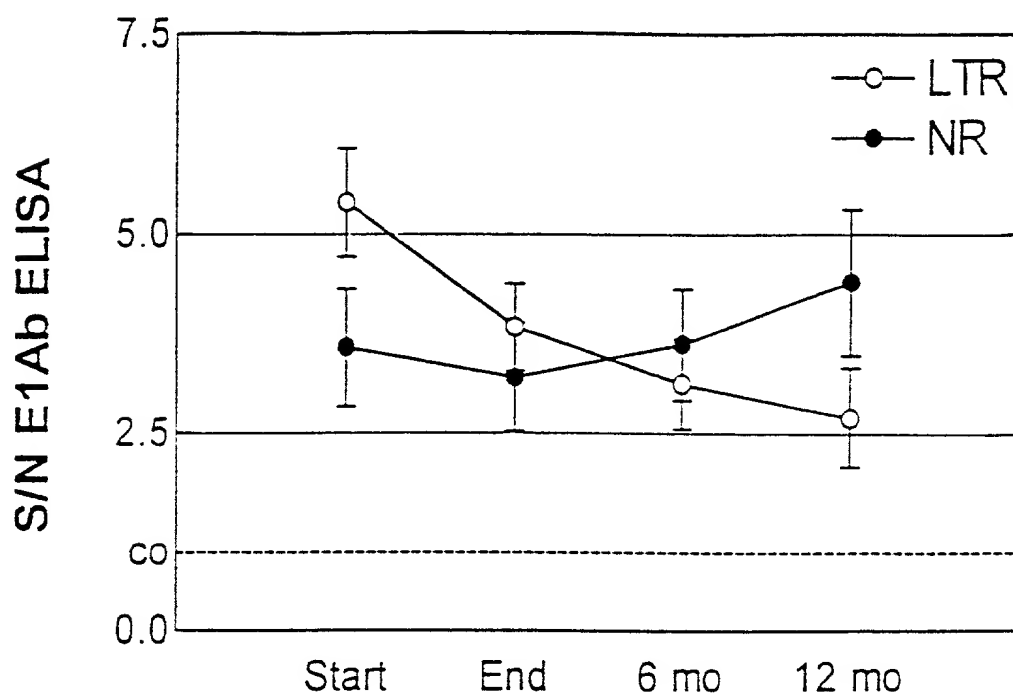
E2 Ab
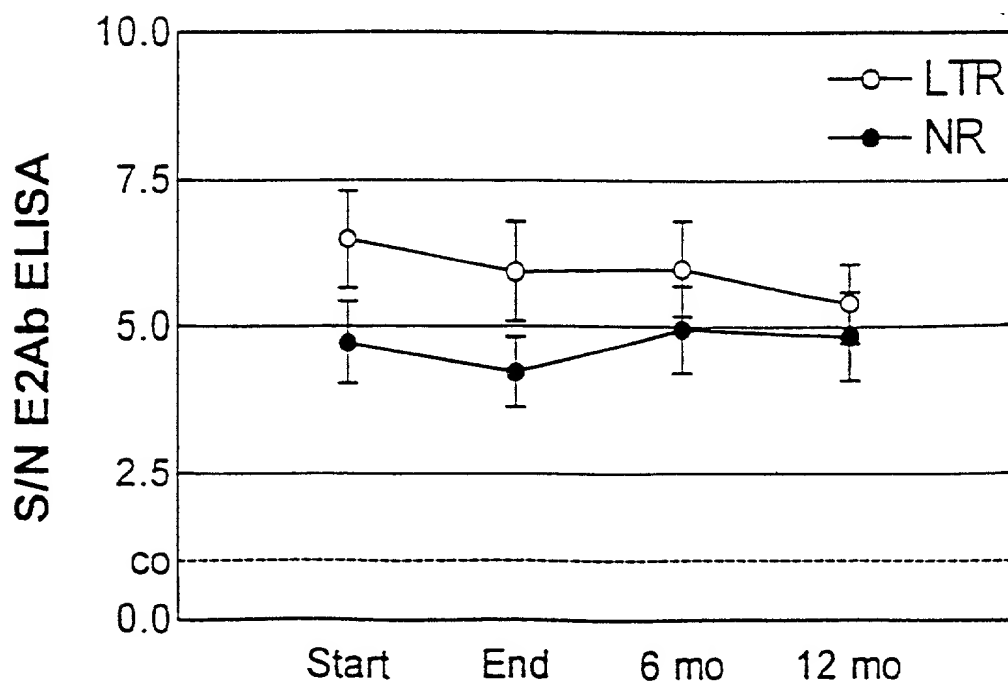
FIGURE 37
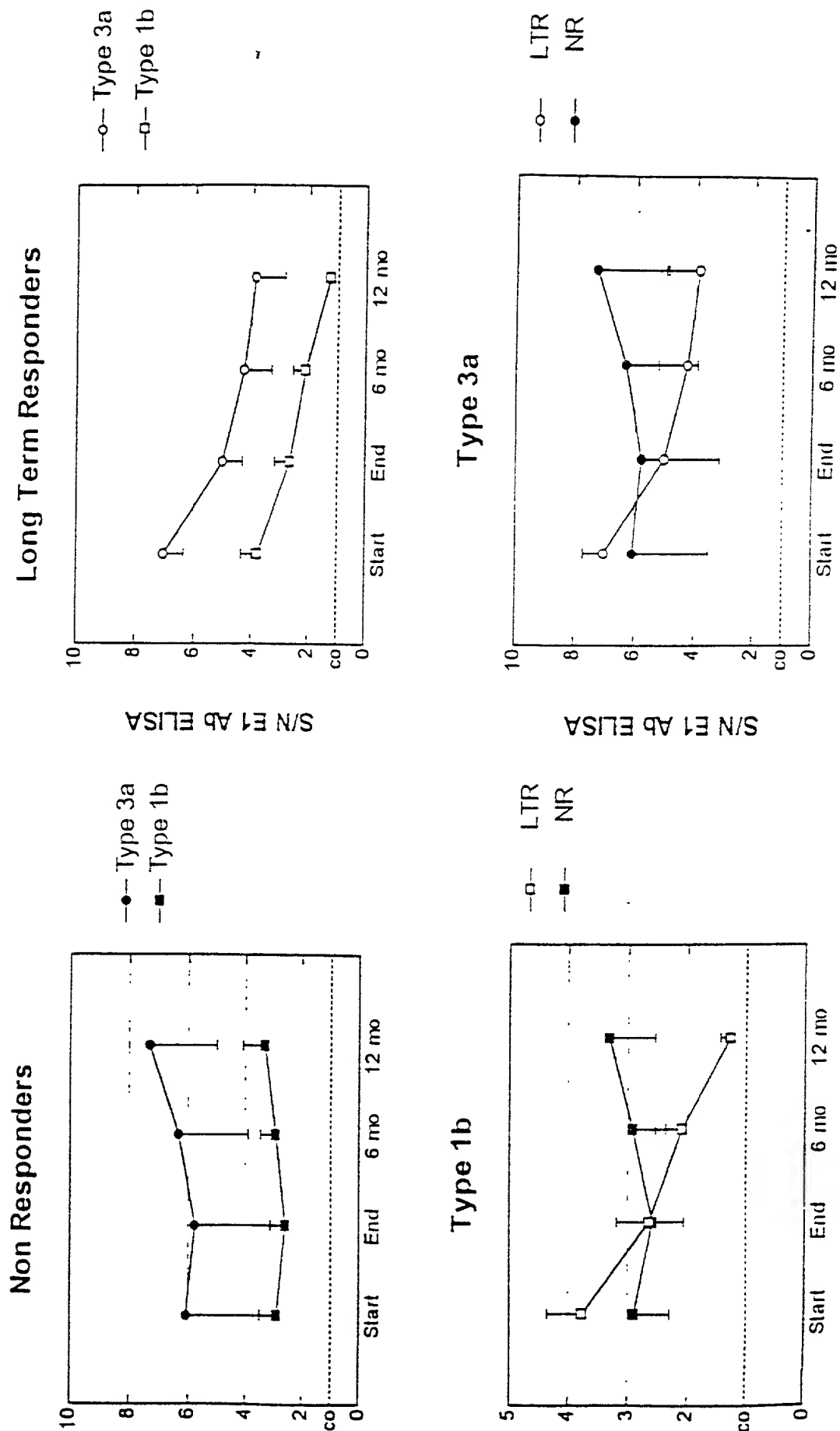
Figure 38

Relative Map Positions of anti-E2 monoclonal antibodies
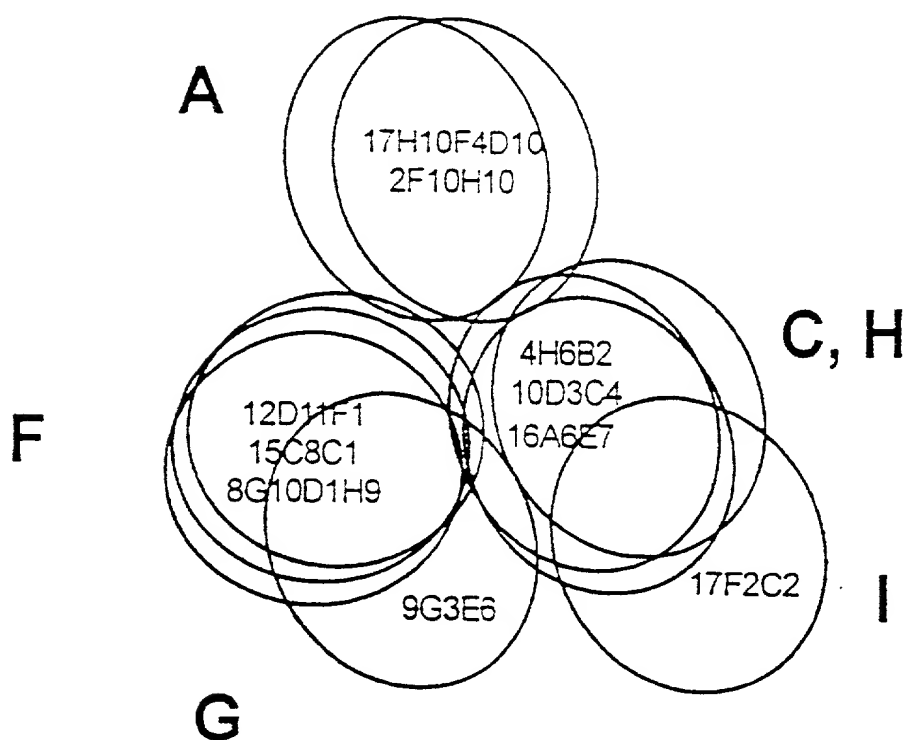
PARTIAL DEGLYCOSYLATION OF HCV E1 ENVELOPE PROTEIN
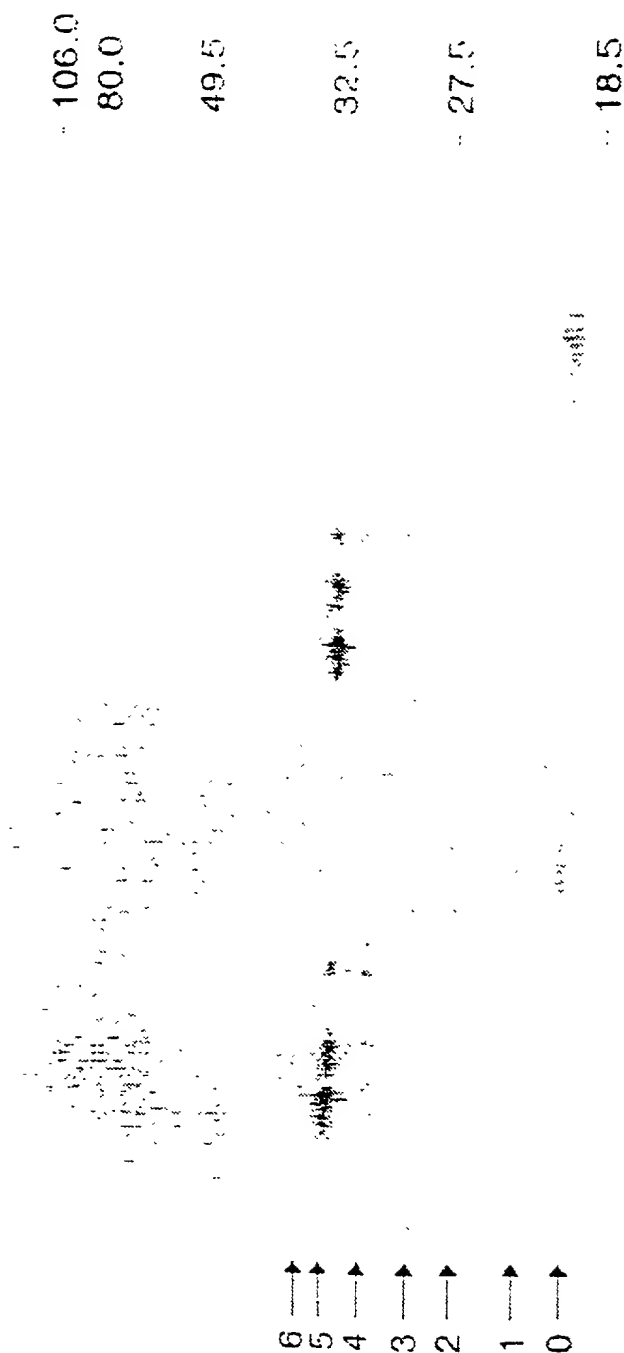
Figure 39

PARTIAL TREATMENT OF HCV E2\E2s ENVELOPE PROTEINS
BY PNGase F
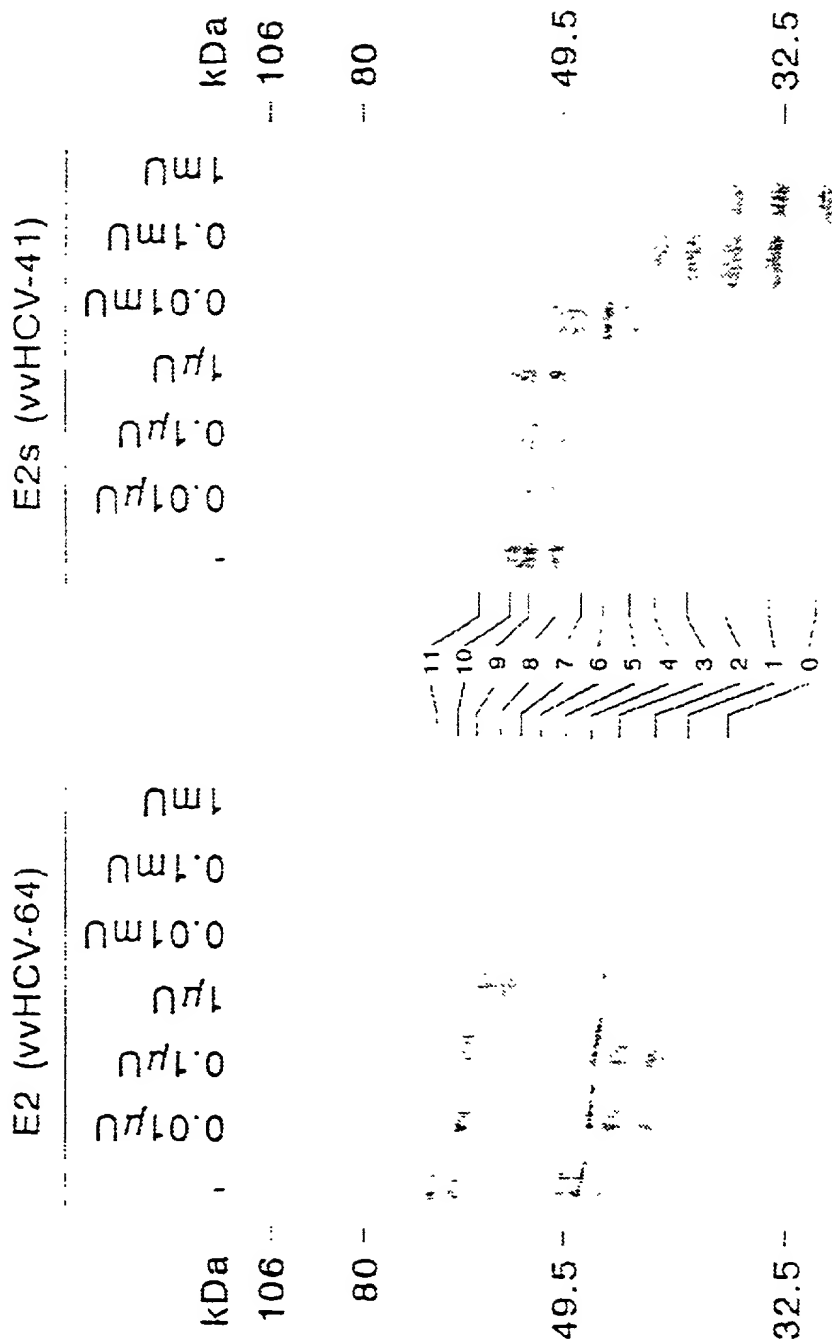
Figure 40
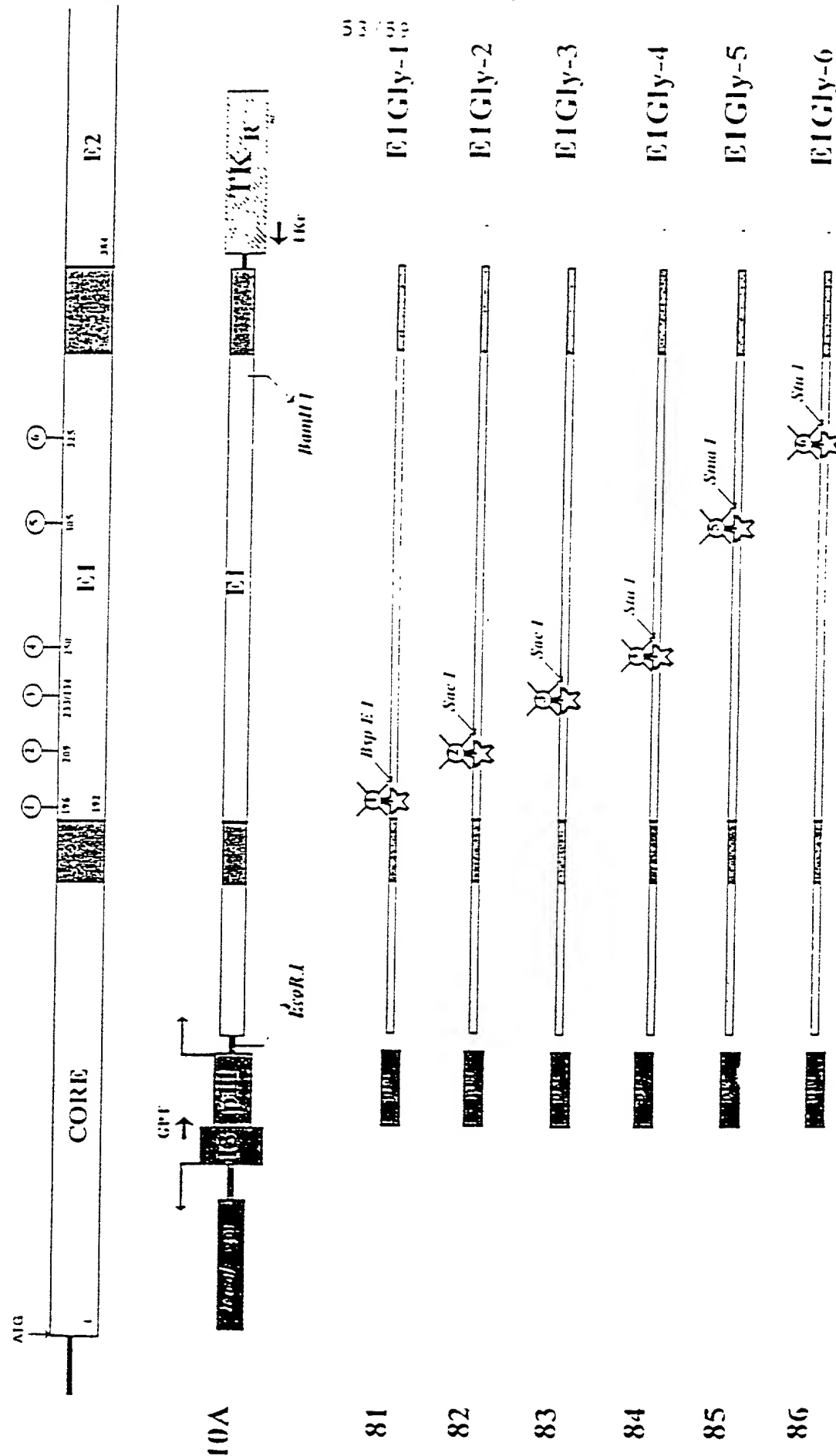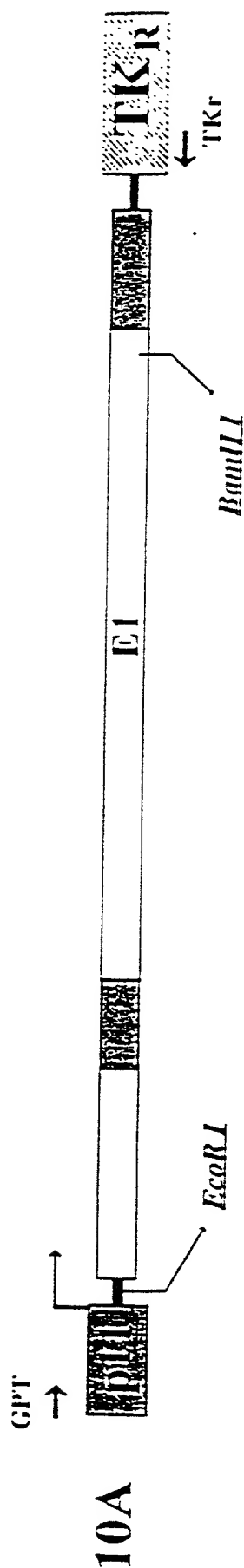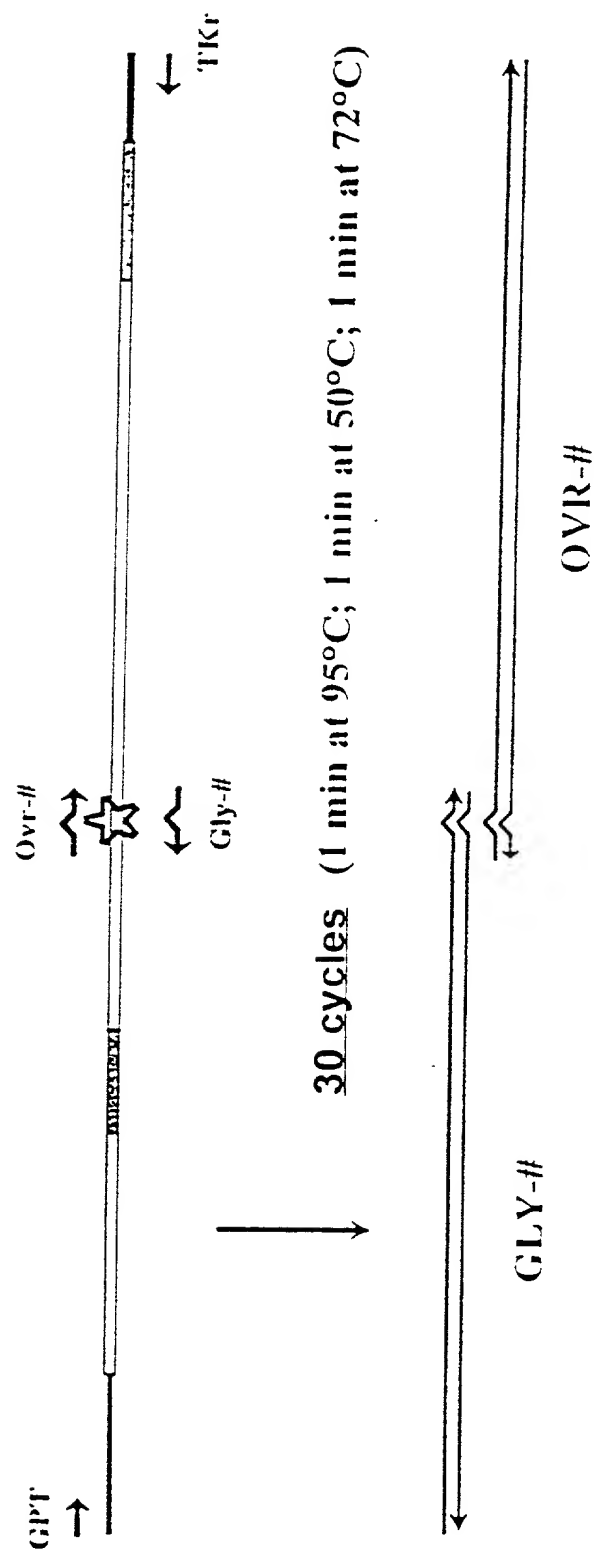
Fig. 41

In Vitro Mutagenesis of HCV E1 glycoprotein

Fig. 42A   *In Vitro* Mutagenesis of HCV E1 glycoprotein

10A

1. First step of PCR amplification   (Gly-# and Ovr-# primers)

30 cycles   (1 min at 95°C; 1 min at 50°C; 1 min at 72°C)

Fig. 42B

2. Overlap extension and nested PCR

a. Overlap extension

GLY-# sense strand

OVR-# antisense strand

2 cycles (1 min at 95°C; 1min at 50°C; 1 min at 72°C)
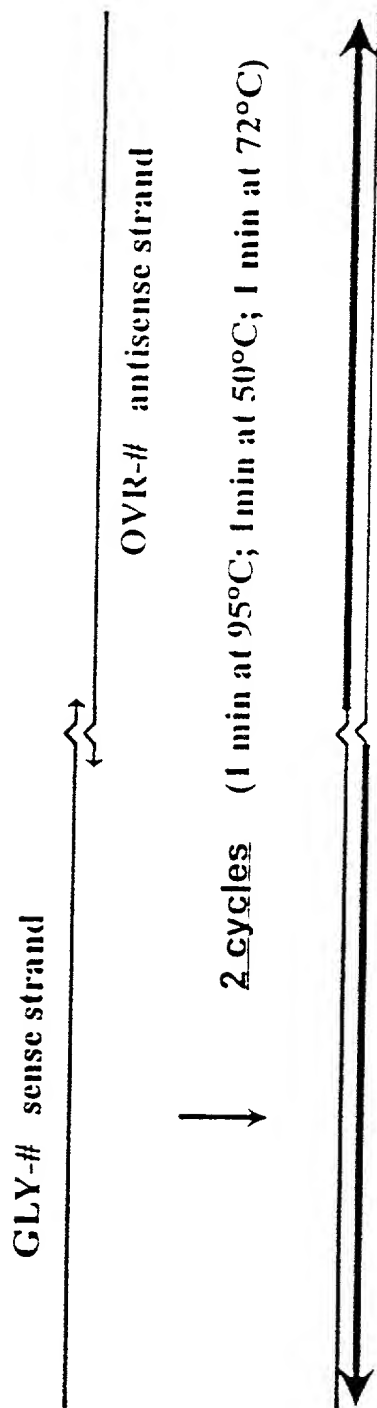
b. Nested PCR amplification (GPT-2 and TKr-2 primers)

GPT-2

TKr-2

25 cycles (1 min at 95°C; 1min at 55°C; 1 min at 72°C)

E1Gly-#

Bam HI

EcoRI

Fig. 43   *In Vitro* Mutagenesis of HCV E1 glycoprotein

| HeLa cells | | | | | | | | | RK 13 cells | | | | | | | | |

Figure 44A

Figure 44B

Figure 45

kDa

119 —
67 —
43 —
29 —
18 ⋮

Figure 46